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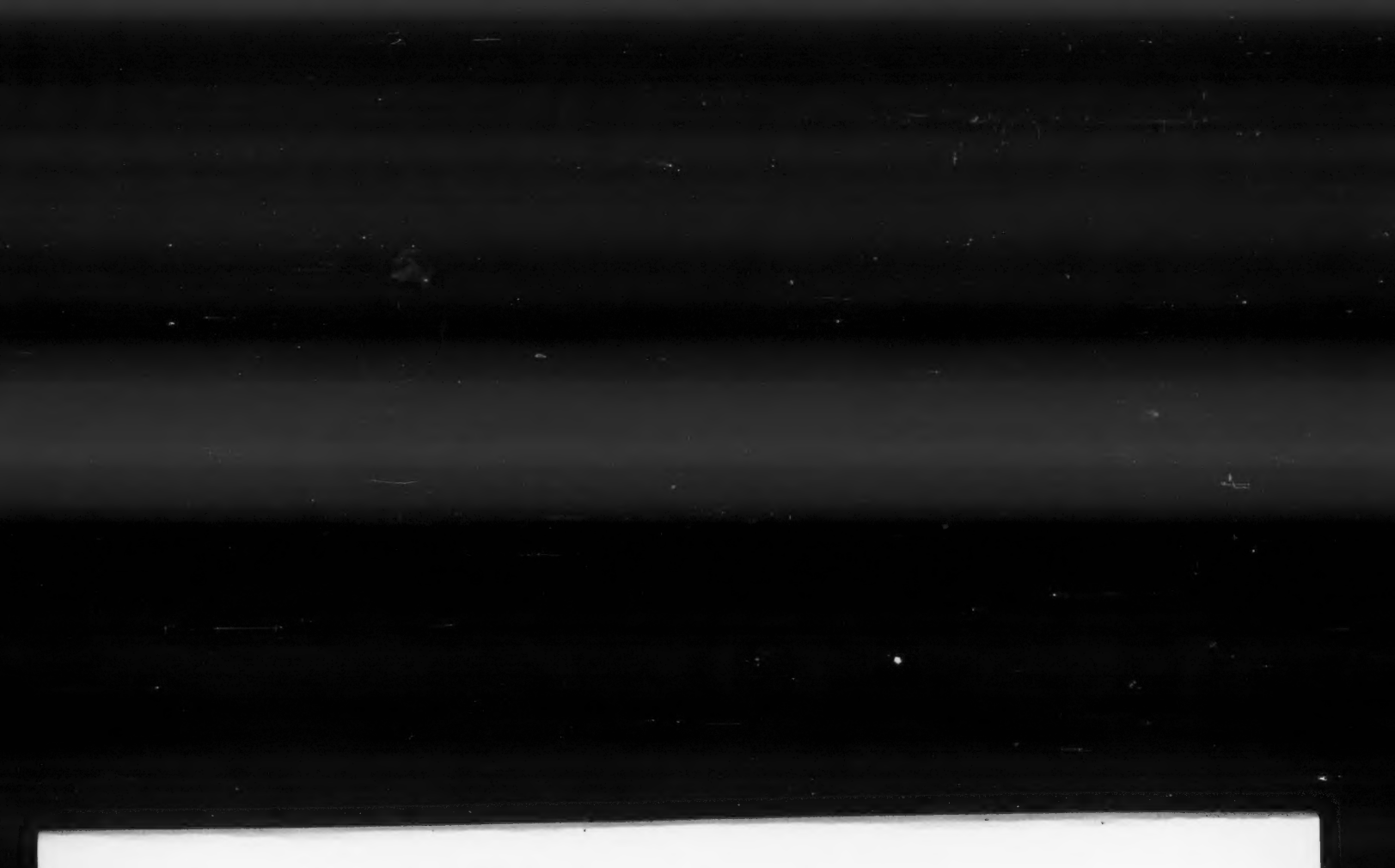

Department of Physiological Chemistry, University of Lund, Lund, Sweden.

On the Excretion of Bile Acid Derivatives in Feces of Rats fed Cholic Acid-24-^{14}C and Chenodesoxycholic Acid-24-^{14}C.

Bile Acids and Steroids 19.

By

SVEN LINDSTEDT and ARNE NORMAN.

Received 19 November 1954.

It has been demonstrated that bile acids are the main metabolic products excreted in the bile of rats fed cholesterol-4-^{14}C (BYERS and BIGGS 1952, BERGSTRÖM 1952, SIPERSTEIN, JAYKO, CHAIKOFF and DAUBEN 1952). The bile acids were identified as taurocholic acid and taurochenodesoxycholic acid (BERGSTRÖM and SJÖVALL 1954). These acids account for more than 95 per cent of the activity found in the saponifiable fraction of the bile. When the acidic steroids present in the feces of rats fed labelled cholesterol were investigated, it was found, however, that only a minor part of the total radioactivity was present as conjugated bile acids. The rest was divided between several groups of substances, which could be separated by chromatography (BERGSTRÖM and NORMAN 1953). The splitting of the peptide bond and the further modification of the bile acids which occur during the intestinal passage, are mainly the result of the action of microorganisms (NORMAN 1955).

The aim of the present investigation was to study whether the products found on chromatograms of the fecal acidic products obtained after feeding cholesterol-4-^{14}C can be found on chromatograms after feeding cholic and chenodesoxycholic acid-24-^{14}C.

The action of microorganisms on bile acids *in vitro* as well as *in vivo* has been studied by several authors (see SOBOTKA 1937, 1943). In 1942, SCHMIDT and HUGHES found that cholic acid is rapidly metabolized by the content of the coecum of guinea-pigs. They measured the disappearance of cholic acid injected into the isolated coecum; after 48 hours of incubation only about twenty per cent of the acid remained unchanged. SCHMIDT, HUGHES, GREEN and COOPER (1942) showed that cholic acid added to cultures of a strain of *Alkaligenes faecalis* isolated from human feces, was converted into different ketocholanic acids among which they isolated 3,7,12-triketocholanic acid. MORIMOTO and SHIMIZU (1951) found that 3,12-dihydroxy-7-ketocholanic acid is formed when cholic acid is incubated with a culture of *Bact. coli comm.* In the last few years extensive work has been done on microbiological hydroxylation of steroids. For a review, see PETERSON (1953).

Experimental.

The labelled bile acids used in these investigations were prepared by BERGSTRÖM, ROTTENBERG and VOLTZ (1953). 1—3 mg was brought into solution as sodium salt in 1—2 ml of 0.9 per cent sodium chloride and injected intraperitoneally into white male rats of the institute stock (weight about 150 g). The rats were kept in metabolism cages and the feces and urine collected daily into 80 per cent ethanol for two weeks. The animals had free access to their normal diet consisting mainly of oats and bread.

The feces from each 24-hour period were crushed and boiled twice with 80 per cent ethanol for three hours. The filtrate was transferred to a volumetric flask and an aliquot evaporated to dryness on an aluminium planchet for determination of radioactivity. A few micrograms of a bile acid with high specific activity was added and the total activity determined. In this way a correction for self-absorption was obtained. This was usually not more than 5—10 per cent of the true activity.

The remaining ethanol extract was evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of water acidified to pH 1 with hydrochloric acid and extracted three times with two volumes of butanol. The combined butanol extracts were washed with water to remove excess hydrochloric acid and then evaporated *in vacuo*. The remaining substance was dissolved in 70 per cent ethanol and subjected to a three stage counter-current distribution to remove lipid material that was not bile acids (cf. NORMAN 1955).

The residue from the combined aqueous phases was then fractionated with the reversed-phase partition chromatographic technique described

for bile acids by BERGSTRÖM and SJÖVALL (1951), SJÖVALL (1953) and NORMAN (1953). The fecal extract containing the bile acids was first chromatographed with solvent system C suitable for separation of conjugated bile acids.

Phase system C. 150 ml of methanol, 150 ml of distilled water, 15 ml of isooctanol and 15 ml of chloroform were mixed in a separatory funnel. 4 ml of the lower phase was used per 4.5 g of hydrophobic Supercel.

The material that remained in the stationary phase was eluted with chloroform and rechromatographed with phase system A.

Phase system A. 180 ml of methanol, 120 ml of distilled water, 45 ml of chloroform and 5 ml of heptane were mixed in a separatory funnel. 4 ml of the lower phase was used per 4.5 g of hydrophobic Supercel.

The fractions were collected by a time-operated fraction collector. Each fraction was titrated with 0.02 N sodium hydroxide in methanol. The solvent was then evaporated off in an oven at 100 °C. The material was redissolved in a small amount of 50 per cent ethanol and evaporated to dryness on copper planchets for determination of radioactivity.

Results.

Figures 1 and 2 show the excretion of activity for each 24-hour period in per cent of administered cholic and chenodesoxycholic acid. These figures also show the cumulative amount of activity excreted.

Figures 3 and 6 show the chromatograms obtained with phase systems C and A of the metabolites of cholic acid in the feces from the second to the fourth day. Figures 4 and 7 show the chromatograms with the same systems of the metabolites of chenodesoxycholic acid in the feces from the second day. For comparison we also reproduce two chromatograms of the acidic products in the feces on the seventh day after feeding cholesterol-4-^{14}C (BERGSTRÖM and NORMAN 1953). The solid lines connect titration values and the open circles connected by broken lines show the c.p.m. of each sample.

Depending on the amount of substance, the size of the column had to be varied. It has been found that the amount of solution necessary to elute a certain band is directly proportional to the size of the column. To facilitate the comparison between different curves the figures on the horizontal axis show the number of ml effluent which would be needed on a standard column made up of 4.5 g of Supercel. To obtain the actual amount of eluting solution used, one has to multiply with the factor given in brackets for each curve.

The titration curve obtained after chromatography of the

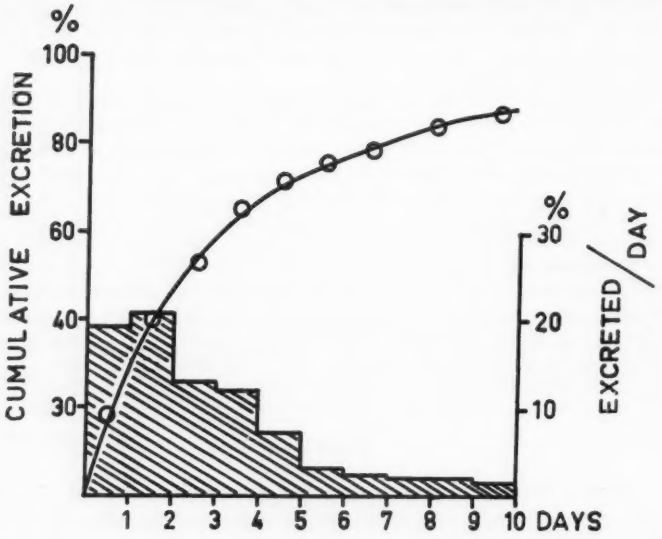

Fig. 1. Isotope excreted in the feces following intraperitoneal administration of cholic acid-24-^{14}C. Shaded areas represent the activity excreted each day in per cent of dose administered; open circles indicate the total amount excreted.

butanol extract of feces is of little significance as the feces contain many acidic products other than bile acids. The greater part of these compounds leave the column with or just after the front with solvent system C. Almost regularly, a distinct titration peak is seen at 130—160 ml in phase system A (this peak is helpful for orientation on the chromatogram). The total amount of these acidic compounds varies with the amount of feces in a 24-hour period.

Discussion.

Amount excreted: It is evident from figures 1 and 2 that carbon atom 24 of the bile acid is completely excreted in the feces. It has previously been shown by BERGSTRÖM, SJÖVALL and VOLTZ (1953), BERGSTRÖM, ROTTENBERG and SJÖVALL (1953) that only an insignificant amount of the activity appears in the expired carbon dioxide after administration of [24—^{14}C] labelled bile acids. In the course of our investigation we never found any

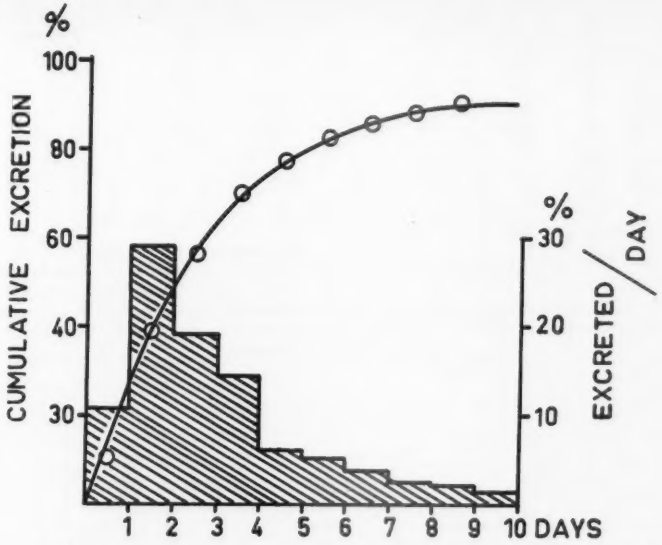

Fig. 2. Isotope excreted in the feces following intraperitoneal administration of chenodesoxycholic acid-24-^{14}C. For explanation, see fig. 1.

measurable activity in the urine. For a further discussion of the rate of elimination, see the paper by LINDSTEDT and NORMAN (1955).

Hydrolysis of the peptide bond: Cholic acid and chenodesoxycholic acid are excreted in the bile, completely conjugated — 1—2 per cent with glycine and the rest with taurine. On the chromatograms with solvent system C (figures 3 and 4), the taurine conjugates all appear in the front, glycocholic acid at 30—50 ml and glycochenodesoxycholic acid at 100—130 ml. It is seen that only a small amount of the acids remain in the conjugated form. A marked splitting of the peptide bonds thus takes place during the intestinal passage. Recent work by NORMAN (1955) has shown that the intestinal microorganisms are mainly responsible for the hydrolysis as practically no splitting of the peptide bonds takes place in rats treated with antibacterial agents. The hydrolysis appears to take place only in the lower part of the intestinal tract as SJÖVALL (1955) did not find any free acids in the content of the small intestine.

The proportion between free and conjugated forms present in

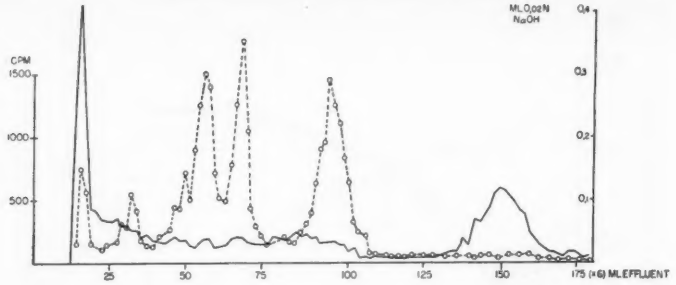

Fig. 3. Chromatographic separation of fecal acids from second to fourth day after administration of cholic acid-24-^{14}C. Phase system C, see page 3. Column: 27 g of hydrophobic Supercel.

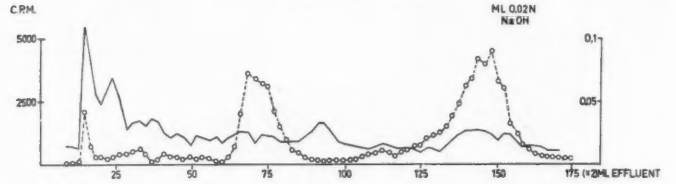

Fig. 4. Chromatographic separation of fecal acids from second day following administration of chenodesoxycholic acid-24-^{14}C. Phase system C, see page 3. Column: 9 g of hydrophobic Supercel.

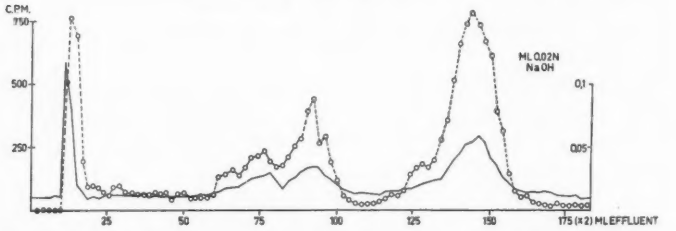

Fig. 5. Chromatographic separation of fecal acids from seventh day following administration of cholesterol-4-^{14}C. Phase system C, see page 3. Column: 9 g of hydrophobic Supercel.

the feces was observed to vary individually in the rat, and in one animal as much as one third of the total activity appeared in the "taurine" band.

Metabolites of cholic acid: It has been shown that cholic acid is not transformed into any new metabolites by the liver (Bergström, Rottenberg and Sjövall 1953). The products separated on the chromatograms shown in figures 3 and 6, result, therefore,

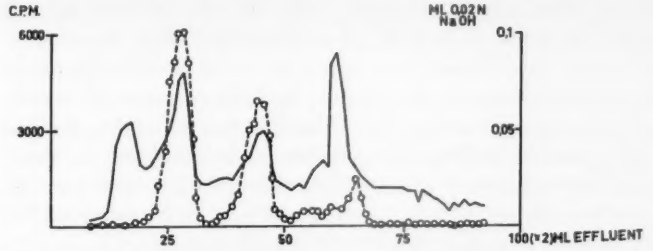

Fig. 6. Cholic acid-24-^{14}C i.p. Chromatographic separation of less hydrophilic acids remaining in stationary phase, fig. 3. Phase system A, see page 3. Column: 9 g of hydrophobic Supercel.

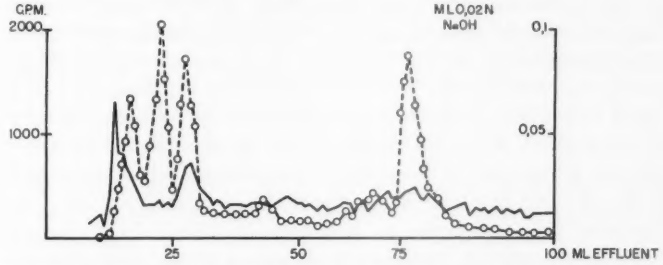

Fig. 7. Chenodesoxycholic acid-24-^{14}C i.p. Chromatographic separation of less hydrophilic acids remaining in stationary phase, fig. 4. Phase system A, see page 3. Column: 4.5 g of hydrophobic Supercel.

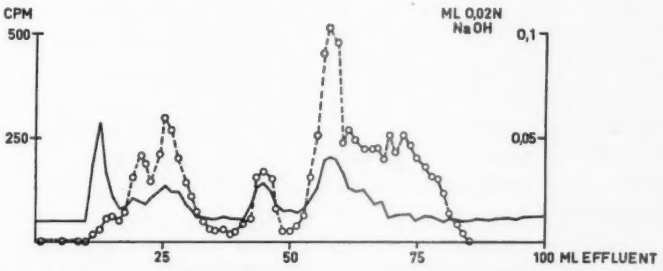

Fig. 8. Cholesterol-4-^{14}C i.p. Chromatographic separation of less hydrophilic acids remaining in stationary phase, fig. 5. Phase system A, see page 3. Column: 4.5 g of hydrophobic Supercel.

from the action of enzymes present in the microorganisms. At the position of free cholic acid (80—110 ml), a distinct peak is seen. This band was hydrolyzed and rerun on the same solvent

system with unlabelled cholic acid. All the labelled material moved with the cholic acid. It is noteworthy that this peak accounts for only 10 to 20 per cent of the total activity. At 50—75 ml, at least two peaks of a more hydrophilic character than cholic acid are regularly seen. More than 50 per cent of the total activity remained in the stationary phase in the run shown in figure 3. The chromatogram of this material with system A is seen in fig. 6 and shows three distinct peaks. The one at 30 ml corresponds to the position of a dihydroxycholanic acid.

When chromatograms of material from feces collected on different days are compared the relative sizes of the bands are found to remain fairly constant during the period studied.

Metabolites of chenodesoxycholic acid: When labelled chenodesoxycholic acid is given to rats with a bile fistula, most of the acid is excreted as taurochenodesoxycholic acid, but about 25 per cent is transformed into two more hydrophilic compounds, none of which is identical with cholic acid after hydrolysis (BERGSTRÖM and SJÖVALL 1954). A chromatogram of the metabolites in feces shows that the major part of the activity is distributed within two bands at 60—70 and 110—140 ml. After alkaline hydrolysis the bands appear at the same positions, indicating that they do not contain conjugated material. As these bands appear at the same positions as those found on chromatograms of hydrolysed material from fistula bile, it is not possible to decide to what extent they contain metabolites formed by the action of microorganisms.

When chromatograms were run on material collected on different days, one animal showed a decrease of the first band with time and a corresponding increase of the second band.

About 30 per cent remained in the stationary phase in the run shown in figure 4. The chromatogram of this material with system A is seen in figure 7. It presents a complicated mixture of different compounds; only a small band appears at the position of chenodesoxycholic acid.

Comparison of the chromatographic patterns obtained after feeding cholic acid, chenodesoxycholic acid and cholesterol.

Chromatograms with solvent system C: The most striking feature is the peak around 150 ml, which is present after feeding cholesterol and chenodesoxycholic acid but regularly absent after feeding cholic acid. At the present time it is not possible to decide whether this peak is homogeneous and contains identical substances in the two cases. The complex at 60—110 ml

seen on the "cholesterol" chromatograms corresponds to peaks in the same position on the "cholic and chenodesoxycholic" chromatograms.

Chromatograms with solvent system A: The complex around 25 ml on the cholesterol chromatogram corresponds to several peaks on the cholic and chenodesoxycholic chromatograms. In the interval 50—75 ml, bands are seen in all chromatograms. The most distinctive feature is the activity peak at 40 ml, accompanied by a titration peak which is seen on the cholesterol and cholic acid chromatograms but which is absent from the chenodesoxycholic chromatogram.

All the bands on the chromatograms of fecal acidic products obtained after feeding cholesterol, can thus be found on the chromatograms after feeding cholic and chenodesoxycholic acid. There are, however, large quantitative variations in different rats of our colony.

Summary.

Cholic acid-24-^{14}C and chenodesoxycholic acid-24-^{14}C were given intraperitoneally to rats and the excretory products in the feces were studied:

1. The given amount is practically completely recovered in feces within 10 days.

2. The acidic products in the feces have been separated by partition chromatography. Only minor amounts were found in the conjugated form. The complicated patterns indicate extensive changes on the bile acid molecule by microbial enzyme systems.

3. The different bands observed on the chromatograms of fecal material after administering labelled cholic and chenodesoxycholic acid together show the same bands that are observed after administration of ring labelled cholesterol.

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References.

Bergström, S., Proc. Roy. Physiogr. Soc. Lund. 1952. *22*. No. 16. — M. Rottenberg and J. Voltz, Acta Chem. Scand. 1953. *7*. 481.

BERGSTRÖM, S., M. ROTTENBERG and J. SJÖVALL, Z. physiol. Chem. 1953. *295*. 278.
— J. SJÖVALL and J. VOLTZ, Acta Physiol. Scand. 1953. *30*. 22.
— and J. SJÖVALL, Acta Chem. Scand. 1954. *8*. 611.
— — Ibidem. 1951. *5*. 1267.
— and A. NORMAN, Proc. Soc. Exp. Biol. N. Y. 1953. *83*. 71.
BYERS, S. O. and M. W. BIGGS, Arch. Biochem. Biophysics. 1952. *39*. 301.
LINDSTEDT, S. and A. NORMAN, Acta Physiol. Scand. 1955. To be published.
MORIMOTO, S. and T. SHIMIZU, Collected papers of the Hiroshima Medical School. 1950. *2*. 9.
NORMAN, A., Acta Chem. Scand. 1953. *7*. 1413.
— Acta Physiol. Scand. 1954. *32*. 1.
— Ibidem. 1955. *33*. 99.
PETERSON, D. H., Research (London). 1953. *6*. 309.
SCHMIDT, L. H., H. B. HUGHES, M. G. GREEN and E. COOPER, J. Biol. Chem. 1942. *145*. 229.
— and H. B. HUGHES, Ibidem. 1942. *143*. 771.
SIPERSTEIN, M. D., M. E. JAYKO, I. L. CHAIKOFF and W. G. DAUBEN, Proc. Soc. Exp. Biol. N. Y. 1952. *81*. 720.
SJÖVALL, J., Acta Physiol. Scand. 1953. *29*. 232.
— Ibidem. 1955. To be published.
SOBOTKA, H., Physiological chemistry of the bile. Baltimore. 1937.
— and E. BLOCH, Ann. Rev. Biochem. 1943. *12*. 45.

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On the Innervation of the Submaxillary Gland Cells in Cats.

By

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Received 29 December 1954.

The investigator of problems concerning the salivary glands will often find the interpretation of experimental observations difficult owing to our defective knowledge of the innervation of the salivary gland cells. It is true that it is well known that secretion can be evoked from a salivary gland by stimulation either of parasympathetic or sympathetic nerve fibres; but whether the individual gland cell can be reached by both types of nerves has been a matter of dispute since the days of Heidenhain and Langley. Among more recent investigators in this field RAWLINSON (1933, 1935) holds the view that in the submaxillary gland of the cat the "alveolar cells" are innervated by the chorda tympani, the "demilune cells" by the sympathetic fibres. LANGENSKIÖLD (1941) found electrophysiological evidence to show that "all secretory cells in the submaxillary gland that are innervated by the sympathetic are also innervated by the chorda". BABKIN in his monograph (1950) maintains that the serous cells have a sympathetic, the mucous cells a parasympathetic innervation (for references and discussion see LANGENSKIÖLD 1949, HILLARP 1949, BABKIN 1950).

MACINTOSH and RAWLINSON (1935) studying the "augmented salivary secretion" suggested that the chemical transmitters of the nerve impulses might diffuse from one set of cells to another. From a physiological point of view it should be of interest to investigate the cells which can be reached by the transmitter

and respond to it disregarding the question whether they are all anatomically supplied by secretory nerve fibres.

Since sympathetic stimulation in cats usually causes an abundant flow of saliva from the submaxillary gland, the following simple way of investigating the problem under discussion seems possible. During secretion at *maximal* rate caused by chorda stimulation a period of sympathetic stimulation is added. Provided that the sympathetic stimulation is able to throw into activity cells which are not already stimulated by the mediator liberated at the chorda nerve endings one would expect the flow of saliva to increase. Experiments of this type are described in the present paper. In some of the experiments secretion was evoked by injection of drugs instead of nerve stimulation.

In many experiments sympathetic stimulation was found, after a short period of time, to diminish the secretion caused by chorda stimulation. This effect was found to be due to the vasoconstriction elicited by stimulation of sympathetic vasomotor fibres. Experiments carried out to study the effect of reduced blood supply on the rate of secretion are described in a subsequent paper.

Although this retarding effect does not become apparent so early as the secretory effect of sympathetic stimulation it seemed an undesirable complication. An attempt was therefore made to abolish the vasoconstrictor action of sympathetic stimulation with its secretory action preserved. In the dog the constrictor fibres are known to degenerate earlier than the secretory fibres after section of the sympathetic trunk in the neck (Ssinelnikoff 1921), but in cats both types of responses disappear simultaneously (Jurist and Rabinovich 1924). Atropine is known to antagonize the secretory more than the vasoconstrictor effect of sympathetic stimulation, but doses are required which completely abolish the secretion on chorda stimulation. A series of drugs with sympathicolytic action were examined, and it was found that some of them were able to separate secretory and vasoconstrictor effects of sympathetic stimulation without interfering with the action of the chorda tympani. Using Priscol which in suitable doses proved to abolish the vasoconstriction but not the secretion it was possible, in the present experiments, to study the flow of saliva on concomitant stimulation of the two secretory nerves without interfering vasoconstriction. In the separate investigation of the effect of vascular changes on the rate of secretion this drug and chlorpromazine which abolishes sympathetic secretion

but not vasoconstriction were found to be useful. A more detailed account of the attempt to find suitable sympathicolytic agents is given in a separate report.

Methods.

The experiments were made on cats under chloralose anaesthesia. The submaxillary duct was cannulated and connected to a bottle containing distilled water. On secretion of saliva small drops of fluid fell from this reservoir. Each drop was recorded by an assistant operating either an electromagnetic signal or in most experiments an ordinate recorder (CLEMENTZ and RYBERG 1949). After injection of heparin the external jugular vein was cannulated, all branches having been tied except that draining the submaxillary gland. The flow of blood was recorded by a photoelectric counter and another ordinate recorder. The blood was then reperfused through a cannula in a femoral vein. The chorda-lingual nerve and the sympathetic trunk were exposed and cut. The peripheral stumps of the nerves were stimulated using 20 stimuli/sec. of supramaximal strength. Drugs were injected through the femoral cannula, through a cannula in the central end of the lingual artery, or into the submaxillary duct as described by EMMELIN, MUREN and STRÖMBLAD (1954).

Results.

On supramaximal stimulation of the chorda tympani the submaxillary gland was often found to be able to secrete in one minute an amount of saliva almost as big as its own weight, and this rate could be maintained for several minutes. The characteristic effect of sympathetic stimulation, on the other hand, is a secretion at successively decreasing rate. Since the rate of flow was not recorded continuously but as intervals between falling drops the size of the maximal rate could only be evaluated in those cases in which several drops were obtained at similar intervals. In most cats this was the case in the beginning of the secretion period; the flow then slowed down and often it ceased completely in spite of continued stimulation. The maximal rate of secretion on sympathetic stimulation was never greater than that elicited by chorda stimulation. It was sometimes as great or almost as great (fig. 2), but in most instances smaller. In one cat, out of about 50 tested, no secretion at all was obtained although the ordinary vasoconstrictor effect of sympathetic stimulation appeared in

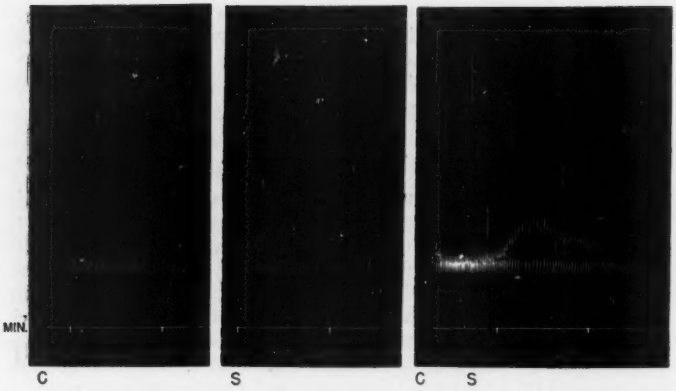

Fig. 1. Cat, 3.2 kg. Records from above: Rate of secretion, with ordinate recorder; time in minutes; signal. First section: chorda, second section: sympathetic stimulation for one minute. Third section: chorda stimulation, starting at C, lasts for the whole period shown. At S sympathetic stimulation is added for one minute.

the gland. Common secretory responses to sympathetic stimulation are shown in figures 1 and 3.

In the experiment of fig. 1 the secretory effects of stimulating the two secretory nerves were first tested separately. The chorda was then again excited and when the rate of secretion had reached a constant level the sympathetic was in addition stimulated for one minute. There was no increase in the rate of flow in spite of the fact that the sympathetic alone had been found to cause an abundant flow of saliva. On the contrary, the flow was found to diminish, to increase again when the sympathetic stimulation ceased. This retarding effect of the sympathetic on the secretion was supposed to be due to the diminished blood supply of the gland caused by the stimulation of sympathetic vasoconstrictor nerves. The degree of retardation of the secretion was found to vary in different experiments; the bigger the vasoconstrictor response, the more secretion slowed down. In some cases sympathetic stimulation did not at all diminish the hyperemia caused by chorda stimulation. No diminution of the secretion was then obtained and it was particularly obvious in those cases that the flow of saliva on chorda stimulation can not be increased by superimposed sympathetic stimulation.

Although it is apparent that the decrease in secretion, when obtained, appears much later than the secretion caused by sym-

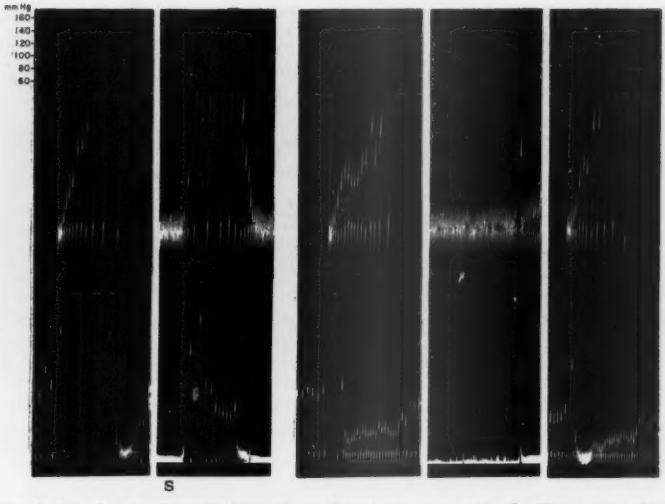

Fig. 2. Cat, 3.1 kg. Records from above: blood pressure; rate of salivary secretion; time in minutes; signal; blood flow through the submaxillary gland. First section: sympathetic stimulation for one minute. During the whole period shown in the second section the chorda was stimulated, and at S sympathetic stimulation for one minute started. Third section starts 20 minutes after the intravenous injection of 5 mg/kg Priscol. The effect of sympathetic stimulation is illustrated. Fourth section starts five minutes after end of section three. During continuous chorda stimulation the sympathetic was stimulated for one minute as in section two (at the end of the sympathetic period the chorda stimulation was switched off for some seconds by mistake). Between section five, showing the effect of sympathetic stimulation, and section four, there is an interval of about five minutes.

pathetic stimulation it was considered an undesirable complication and attempts were made to eliminate it. By reducing or abolishing the vasoconstrictor action of sympathetic stimulation by injection of Priscol this proved possible.

Fig. 2 shows such an experiment. Sympathetic stimulation caused a pronounced secretion and vasoconstriction. When applied during chorda stimulation it diminished the hyperemia considerably and the salivary secretion diminished very much. After Priscol injection only a slight vasoconstriction was obtained on sympathetic stimulation, whereas the secretory response was not diminished. Now sympathetic stimulation did not reduce the hyperemia caused by the chorda. It is obvious that sympathetic stimulation, in spite of its pronounced secretory effect, did not increase the flow of saliva above the level produced by chorda stimulation.

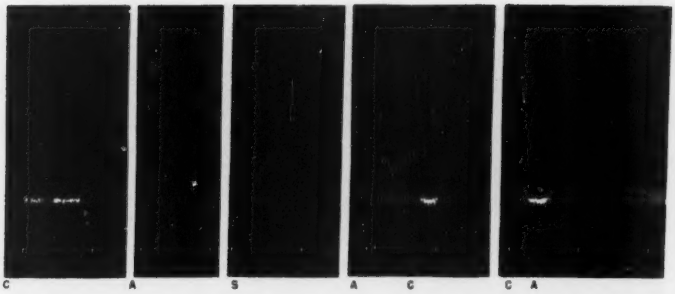

Fig. 3. Cat, 2.8 kg. Records as in fig. 1. C: chorda, S: sympathetic stimulation. A: intravenous injection of adrenaline, the first time 20 μg/kg, then 30 μg/kg.

Some information about the distribution of the secretory nerves in the gland may be gained by studying the effect of drugs, which cause secretion. Such drugs were injected intravenously, close arterially or into the duct in doses which were raised until secretion at a maximal rate was evoked. Fig. 3 illustrates the effect of adrenaline. The quickest flow which could be obtained with adrenaline was much slower than that caused by chorda but equal to that caused by sympathetic stimulation. When the gland, secreting after the injection of a big dose of adrenaline was in addition stimulated via the chorda the rate of flow increased very much, but not above a level for which the chorda alone could be responsible. Injection of adrenaline, on the other hand, could not further increase the rapid secretion going on during chorda stimulation. After a short period of time it reduced the flow in a similar way as sympathetic stimulation.

The maximal rate of secretion evoked by noradrenaline was equal to that caused by adrenaline. The effect of these drugs never exceeded that of sympathetic stimulation. When sympathetic stimulation caused a slow secretion only, this was found to be the case with adrenaline and noradrenaline also, and both were devoid of secretory action in the solitary case in which sympathetic stimulation did not induce any salivary secretion.

Acetylcholine produced a secretion as rapid as did parasympathetic stimulation. This could best be demonstrated if the drug was given into the duct or through the arterial cannula. The big doses required when the intravenous route was used affected the general circulation so much as to interfere seriously

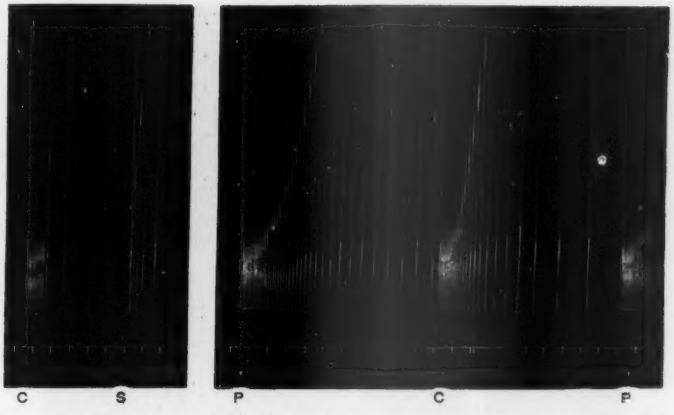

Fig. 4. Cat, 2.7 kg. Records as in fig. 1. C: chorda, S: sympathetic stimulation for one minute. P: intravenous injection of pilocarpine hydrochloride, the first time 100 μg/kg, the second time 200 μg/kg.

with the blood supply of the gland. Carbaminoylcholine and pilocarpine could give a maximum equal to that of acetylcholine and chorda stimulation. The effect of pilocarpine is illustrated in fig. 4. An intravenous dose of 100 μg/kg caused a flow of saliva as quick as that elicited by the chorda, and double the dose could not increase the flow further. When the effect of pilocarpine was wearing off the secretion could be accelerated by chorda stimulation, but only to the maximum characteristic of chorda or pilocarpine separately.

Eserine injected into the duct caused a secretion, the maximum of which equalled that of chorda stimulation. In the experiment of fig. 5 eserine had been given intravenously producing a slow flow of saliva. The figure shows the secretory response to chorda stimulation before and after eserine injection. The maximal rate of secretion was not affected by the eserine; the only effect was a prolonged secretion when the stimulation of the chorda had ceased.

Discussion.

The present experiments were designed to show the extent to which it is possible to excite the submaxillary gland via the

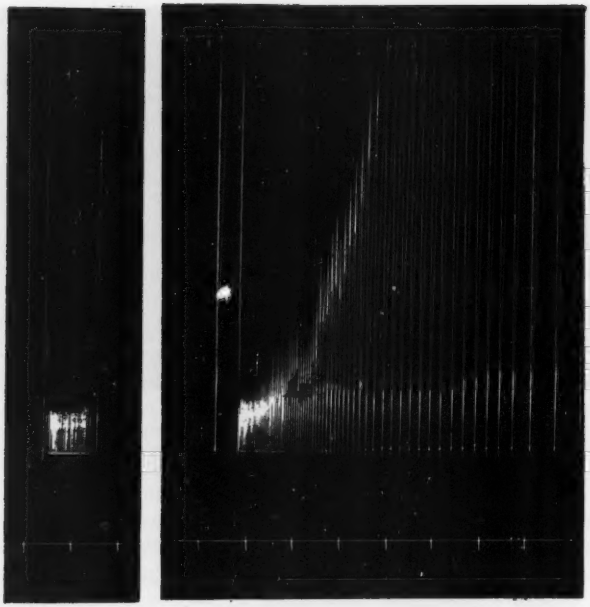

Fig. 5. Cat, 2.5 kg. Records as in fig. 1. C: chorda stimulation for one minute. 15 minutes before the second part of the tracing starts eserine sulphate in a dose of 0.5 mg/kg had been given intravenously.

two secretory nerves, disregarding the question whether a cell excited is supplied by a secretory fibre of its own or reached by diffusing mediators from postganglionic endings in contact with other cells. It is not even out of the question that the mediator might originate from preganglionic parasympathetic fibres in the gland or postganglionic vasomotor fibres. RICHINS and KUNTZ (1953) hold the opinion that the sympathetic nerve carries no secretory fibres to the submaxillary gland cells which, however, may be stimulated by sympathin from the vasoconstrictor fibres.

The rapid flow of saliva elicited by supramaximal stimulation of the chorda tympani cannot in any way be further accelerated. Pilocarpine, acetylcholine and carbaminoylcholine are capable of producing a flow of the same, but not a higher rate. It seems reasonable to assume that the chorda fibres are able to excite all the secretory elements of the gland to maximal activity.

Even if the transmitter of the chorda endings is given the best chances against the limiting action of the cholinesterase by injection of eserine chorda stimulation does not yield a higher maximum than before the administration of eserine.

The maximal rate of secretion produced by sympathetic stimulation, varying in different cats, is always as big as that given by adrenaline and noradrenaline. It may therefore be inferred that the sympathetic, even if it does not contain a single secretory fibre in the true anatomical sense, is able to activate all the secretory elements excitable by adrenaline and noradrenaline. The question whether such maximal stimulation of the sympathetic occurs outside the laboratory need not be considered at present. The finding that sympathetic stimulation does not further increase the flow caused by supramaximal stimulation of the chorda tympani indicates that the sympathetic works on elements which can also be affected by the parasympathetic nerve. Since it is not known whether a cell stimulated via the sympathetic would yield the same amount of saliva as if stimulated via the chorda it cannot be stated that all the secretory cells have a double "innervation", in a physiological sense. From the observation that in some experiments the sympathetic gives a maximum just as high as that obtained by chorda stimulation it is tempting to assume that this may in some instances be the case whereas in others the parasympathetic "innervation" is more complete. At any rate, the results tally obviously better with the conclusion of LANGENSKIÖLD (1941) that "all secretory cells in the submaxillary gland that are innervated by the sympathetic are also innervated by the chorda," than by that defended by BABKIN (1950).

Some further arguments in support of the opinion here advocated may be given. Section of the chorda tympani renders the gland supersensitive not only to acetylcholine and pilocarpine but also to adrenaline. It is unlikely that this implies that cells normally not excitable by adrenaline become sensitive to this agent, since the maximal rate of secretion on adrenaline injection is not higher in the denervated than in the normal gland (EMMELIN and MUREN 1951). As the sympathetic seems to be able to affect all the cells sensitive to adrenaline it may be concluded that section of the chorda affects cells which can be influenced by the sympathetic also. Similarly, removal of the superior cervical ganglion sensitizes the gland not only to noradrenaline but to

acetylcholine and pilocarpine as well. A further indication of such a double influence may be the fact that the depressor agent of saliva appears in the saliva particularly on sympathetic stimulation (FELDBERG and GUIMARAIS 1935) but disappears from the gland after section of the chorda tympani (EMMELIN and HENRIKSSON 1953).

It should be emphasized that the experiments have so far dealt exclusively with the submaxillary gland of the cat. The position as to the "innervation" may be different in other salivary glands and in other species. In addition it ought to be borne in mind that the indicator of secretory activity in the present investigation has been the volume of fluid produced. It may be that the conclusions drawn from the observations of the present experiments do not apply to some types of secretory cells which, although contributing very little to the volume of the saliva, may add some specific components to it. The possibility of a reabsorption of fluid, under nervous control, has not been taken into account in this investigation.

Summary.

The opinion usually given in the literature that in the submaxillary gland of the cat one group of secretory cells is controlled by parasympathetic, another by sympathetic fibres has been tested experimentally.

When the gland is secreting in response to supramaximal stimulation of the chorda tympani sympathetic stimulation does not further accelerate the flow, even if it is able to cause an abundant flow of saliva when given alone.

The results of the experiments do not agree with the current conception of the innervation but support the view of LANGENSKIÖLD (1941), founded on electrophysiological investigations, that "all secretory cells in the submaxillary gland that are innervated by the sympathetic are also innervated by the chorda".

The investigation was supported by a grant from the Swedish Medical Research Council.

Technical assistance was given by Miss Ursula Delfs.

References.

Babkin, B. P., Secretory mechanism of the digestive glands. P. B. Hoeber, New York 1950.

Clementz, B. and C.-E. Ryberg, Acta Physiol. Scand. 1949. *17*. 339.

Emmelin, N. and K. G. Henriksson, Ibidem 1953. *30*. Suppl. 111. 75.

— and A. Muren, Ibidem 1951. *24*. 103.

— A. Muren and R. Strömblad, Ibidem 1954. *32*. 325.

Feldberg, W. and J. A. Guimarais, J. Physiol. 1935. *85*. 15.

Hillarp, N.-Å., Acta Anatom. 1949. *8*. 190.

Jurist, P. M. and B. A. Rabinovich, J. Physiol. 1924. *58*. 274.

Langenskiöld, A., Acta Physiol. Scand. 1941. *2*. Suppl. VI.

MacIntosh, F. C. and H. E. Rawlinson, Quart. J. exp. Physiol. 1935. *25*. 199.

Rawlinson, H. E., Anat. Rec. 1933. *57*. 289.

— J. Anat. 1935. *70*. 143.

Richins, C. A. and A. Kuntz, Amer. J. Physiol. 1953. *173*. 471.

Ssinelnikoff, E. J., J. russe de Physiol. 1921. *3*. 97. Quoted from Rona Berichte ges. Physiol. Pharm. 1923. *15*. 75.

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Blood Flow and Rate of Secretion in the Submaxillary Gland.

By

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In his experiments on the phenomenon of "augmented salivary secretion" LANGLEY (1878) found that minimal effective stimuli applied simultaneously to the chorda and sympathetic nerves evoked a salivary secretion at least equal to the sum of the amounts from separate stimulation; with strong stimuli, on the other hand, the amount of saliva was not only less than the sum, but less than that produced by stimulation of the chorda alone. The latter effect had been observed earlier by other investigators, for instance CZERMAK (1857) and KÜHNE (1866), and taken as evidence to show the existence of a mutual antagonism between the two nerves. LANGLEY, however, considered it more probable that the reduction in the secretion is due to a diminution of the blood supply of the salivary gland caused by stimulation of sympathetic vasoconstrictor fibres. HEIDENHAIN (1868) had favoured this opinion, and later investigators have, likewise, accepted this view (CARLSON and MCLEAN 1908, GESELL 1920).

An investigation of the innervation of the submaxillary gland in cats, reported in the preceding paper, necessitated a study of the relations between secretory rate and blood flow in the gland in some detail. These experiments are described in the present paper.

Methods.

The experiments were made on the submaxillary gland of the cat under chloralose anaesthesia using the same methods as in the previous paper. Care was taken to apply stimuli of supramaximal strength to the nerves.

Results.

In some preliminary experiments the amount of saliva produced during one minute in response to chorda and sympathetic stimulation separately and simultaneously was measured. In a typical experiment the chorda caused a flow of 0.93 cc in one minute, the sympathetic 0.24 cc and the combined stimulation 0.56 cc. Such an effect was, however, not obtained in all experiments. In some, simultaneous stimulation evoked a flow just as big as that produced by chorda stimulation alone; in no experiment a bigger effect was obtained.

When, in later experiments, the secretion and the blood flow were recorded simultaneously, it was found that stimulation of the sympathetic during a period of chorda stimulation in most cases reduced the hyperemia brought about by chorda stimulation, but this was not always the case. A reduction of the rate of secretion was observed only in those cats in which sympathetic stimulation was able markedly to decrease the blood flow. The bigger this effect on the blood flow was, the bigger was the diminution of the secretory rate. No explanation was found for the fact that the sympathetic vasoconstriction was sometimes but not always able to overcome the chorda vasodilatation; it sometimes failed to do so even if the sympathetic vasoconstrictor response was very pronounced.

Sympathetic stimulation causes not only constriction, but in addition a vasodilatation which usually appears after the stimulation period but not infrequently comes to light already during the stimulation period, interrupting the constriction. This dilatation has been attributed to the effect of metabolites, produced during the secretion and accumulated in the constriction period (Barcroft 1907, 1912). By injection of chlorpromazine it is possible to abolish the secretory effect of sympathetic stimulation and at the same time the dilator effect disappears whereas the constrictor effect is preserved (Emmelin 1955). It was thought that the abolition of the vasodilator component of sympathetic stimulation might increase the chances of the sympathetic constriction to reduce the chorda hyperemia. This was in some cats found to be the case. Fig. 1 shows such an experiment. In this cat sympathetic stimulation diminished the chorda hyperemia only slightly, and the rate of secretion was not at all affected.

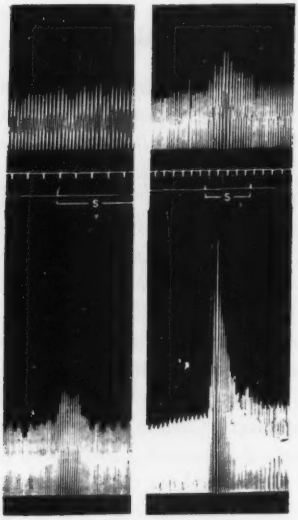

Fig. 1. Cat, 3.0 kg. Records from above: salivary secretion during continuous chorda stimulation; time marks: 10 seconds; signal; blood flow. S: sympathetic stimulation added to the chorda stimulation. About half an hour before the second section of the tracing starts 0.5 mg/kg chlorpromazine (Hibernal Leo) had been injected intravenously.

After the injection of chlorpromazine, however, the blood flow was much more retarded by sympathetic stimulation, and the secretion slowed down correspondingly.

Another sympathicolytic agent, Priscol, was found to antagonize the vasoconstrictor action of sympathetic stimulation more than the secretory effect. When this drug was given in experiments in which sympathetic stimulation strongly reduced the dilator and secretory effects of the chorda it was found that neither hyperemia nor secretion were diminished when the sympathetic stimulation was added. Such an experiment is shown in the second figure of the preceding paper.

In a special series of experiments the effect on the chorda secretion of other measures, which reduce the blood flow through the gland to about the same extent as sympathetic stimulation, was investigated. Various procedures were tried such as arterial and venous occlusion, lowering the blood pressure by vagal stimulation or withdrawal of blood, or injection of vasoconstrictor

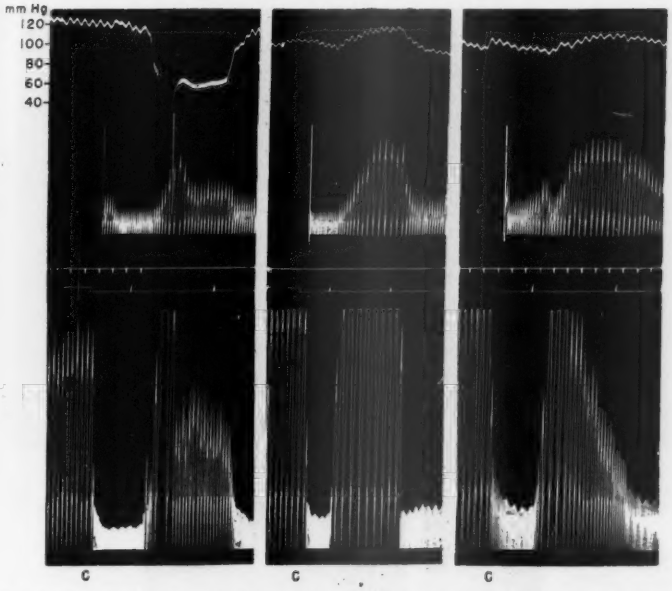

Fig. 2. Cat, 2.6 kg. Records from above: blood pressure; salivary secretion; time in 10 seconds; signal; blood flow. C marks the point where chorda stimulation starts to continue for the rest of the period shown in each section of the tracing. Between the two marks of the signal the peripheral stump of the right vagus was stimulated in the first section; the carotid artery was clamped in the second section; the sympathetic was stimulated in the third section.

agents into the gland. Fig. 2 shows, in the same cat, the effect of stimulation of the peripheral stump of the right vagus in the neck, occlusion of the carotid artery, and sympathetic stimulation. In the experiment of fig. 3 two doses of pitressin were injected through the lingual artery towards the gland. It can be seen in these figures that a reduced blood flow to the gland, irrespective of the method used to produce it, was followed after a few seconds by a retardation of the chorda secretion, more pronounced the bigger and more longlasting the vascular effect. When the increased flow was restored the secretion increased again but the return occurred slowly after a pronounced circulatory obstruction. Occlusion of the vein draining the gland had a similar effect. Bleeding the animal reduced the secretory rate if it was big enough to diminish the blood flow through the gland; when the blood was reperfused the rate of secretion increased again.

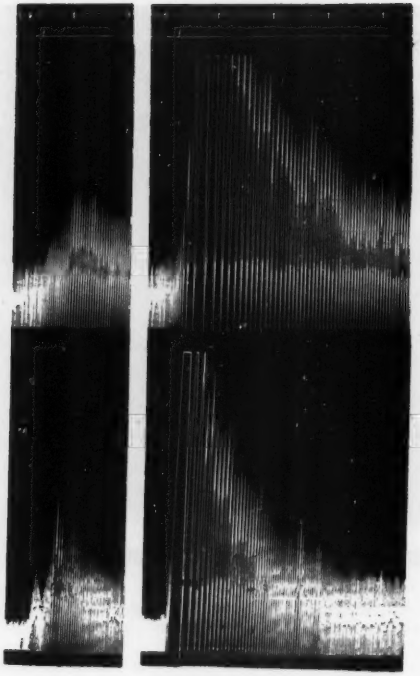

Fig. 3. Cat, 4.1 kg. Records from above: time in minutes; signal; salivary secretion; blood flow. During both periods shown the chorda was stimulated. At the signals injection into a cannula in the central stump of the lingual artery the first time 0.2 I. U., the second time 0.3 I. U. of pitressin.

The experiment of fig. 4 shows the effect on chorda secretion of a vasoconstriction of long duration. The constriction was elicited by injection of a big dose of adrenaline into the submaxillary duct. Two minutes after the injection chorda stimulation could produce a small acceleration of the blood flow only, and no secretion. About ten minutes later the vasoconstriction was still pronounced and the effect of chorda stimulation small. About half an hour after the administration of adrenaline the chorda was able to cause a rapid flow of blood through the gland. The secretory response, however, was far from restored.

It may be added that salivary secretion induced by pilocarpine injection was found to be retarded just as chorda secretion by

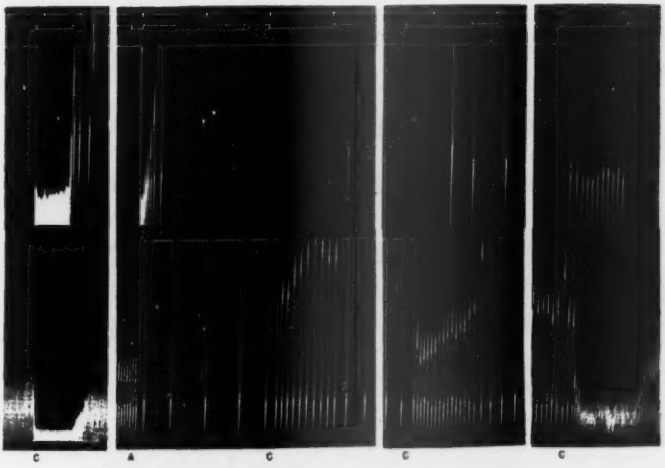

Fig. 4, Cat, 3,2 kg. Records as in fig. 3. C: chorda stimulation, A: injection of 200 μ adrenaline, in 0.2 cc, into the submaxillary duct. See text for details.

procedures which reduced the blood flow through the gland such as arterial occlusion, sympathetic stimulation or injection of adrenaline.

Summary.

The secretion of saliva from the submaxillary gland of the cat which is obtained on supramaximal stimulation of the chorda tympani is usually diminished by simultaneous stimulation of the sympathetic trunk in the neck. The following observations indicate this effect to be a result of the diminished blood supply brought about by stimulation of sympathetic vasoconstrictor fibres. The effect is obtained only in those cases in which the sympathetic stimulation reduces the blood flow and it is bigger the more pronounced this reduction is. It increases when the vasoconstrictor effect is enlarged by the injection of chlorpromazine. It disappears when the vasoconstrictor action is abolished by Priscol. The chorda secretion is correspondingly reduced by procedures which diminish the blood flow through the gland to about the same extent as sympathetic stimulation.

The gland secreting at a maximal rate seems to be relatively

susceptible to reduction of the blood flow, moderate and short-lasting diminution of the intense hyperemia causing a decrease in the rate of secretion.

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Technical assistance was given by Miss Ursula Delfs.

References.

Barcroft, J., J. Physiol. 1907. *35*. 29 P.
— and H. Piper, Ibidem 1912. *44*. 359.
Carlson, A. J. and F. C. McLean, Amer. J. Physiol. 1908. *20*. 457.
Czermak, quoted from Langley, 1878.
Emmelin, N., Acta Physiol. Scand. 1955. *34*. 29.
Gesell, R., Amer. J. Physiol. 1920. *54*. 166.
Heidenhain, R., Stud. des Physiol. Inst. zu Breslau. Leipzig, 1868.
Kühne, W., Lehrbuch der physiol. Chemie, Leipzig, 1866.
Langley, J. N., J. Physiol. 1878. *1*. 96.

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Sympathicolytic Agents Used to Separate Secretory and Vascular Effects of Sympathetic Stimulation in the Submaxillary Gland.

By

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The sympathetic trunk in the neck is known to carry vasoconstrictor fibres to the submaxillary gland in cats. It is also generally assumed to contain secretory fibres for the gland; recently, however, RICHINS and KUNTZ (1953) have expressed the view that the secretion elicited on stimulation of the sympathetic trunk is caused by sympathin diffusing from the vasoconstrictor nerve endings. Sympathetic stimulation produces, apart from secretion and vasoconstriction, further a vasodilatation in the gland, which may sometimes be almost as pronounced as that accompanying chorda stimulation. It usually appears after cessation of the stimulation but sometimes earlier, the constriction thereby diminishing, or periods of constriction and dilatation alternating. According to CARLSON (1908) the dilation is due to stimulation of vasodilator fibres present in the sympathetic trunk. BARCROFT (1907, 1912), on the other hand, regards the dilatation as caused by vasodilator metabolites produced in the secreting gland and accumulated because of the constriction.

In the course of an investigation on the innervation of the submaxillary gland, published in a previous paper (EMMELIN 1955) it became desirable to be able to separate secretory and vasomotor effects of stimulation of the sympathetic nerve. It was thought that the gland cells and the vessels might differ in

susceptibility to a blocking agent, and various drugs with sympathicolytic properties were tested, starting with very small doses of the drugs.

Methods.

Cats under chloralose anaesthesia were used, as in the two preceding papers. Sympathicolytica were injected intravenously, or sometimes into the submaxillary duct or from a cannula inserted in the lingual artery. The drugs studied were: Dihydroergotamine, Chlorpromazine, Priscol, Antistin and Regitin.[1]

Results.

Dihydroergotamine. When increasing doses of this drug were tried, the secretory, vasoconstrictor and vasodilator effects of sympathetic stimulation were found to diminish concomitantly.

Chlorpromazine. A sympathicolytic quality has been attributed to this drug by various authors (see POCIDALO et al. 1952, COURVOISIER et al. 1953). The present experiments showed that small doses of this drug are able to abolish the secretory and vasodilator responses to sympathetic stimulation without affecting the vasoconstriction. The smallest effective dose was of the order of 0.005 mg/kg, given intravenously. With 0.1—0.2 mg/kg the secretory effect was completely abolished. The dilatation was correspondingly reduced, and with secretion absent there was no dilatation. The vasoconstrictor action, on the other hand, was usually not at all diminished by these doses. It appeared, on the contrary, often more pronounced when not counteracted by a dilator component. Bigger doses decreased the vasoconstrictor response also. After injection of 2—5 mg/kg the vasoconstriction was usually completely or almost completely abolished, but in a few experiments a slight constrictor action of sympathetic stimulation could be observed even after as much chlorpromazine as 10—20 mg/kg.

In order to get a distinct separation of the secretory and the constrictor responses it was often found suitable to wait for 15—30 minutes after the injection. With small doses the secretory antagonism developed slowly, increasing during the first 15 minutes. The effect appeared quicker with bigger doses, but the

[1] The chlorpromazine preparation (Hibernal Leo) was kindly supplied by A. B. Leo and the Regitin by the Ciba A. G.

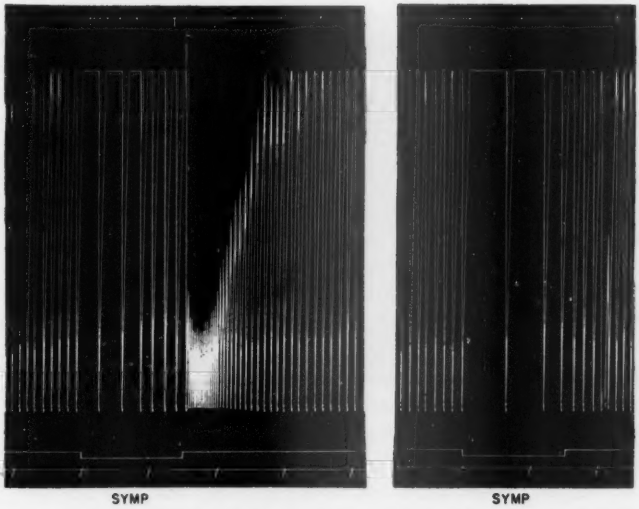

Fig. 1. Cat, 3.3 kg. Records from above: salivary secretion, blood flow, signal, time in minutes. Symp.: stimulation of the sympathetic trunk in the neck for $1^1/_2$ min. The cat had received 1 mg of atropine sulphate intravenously at the beginning of the experiment. 15 minutes before the second stimulation 0.3 mg/kg of chlorpromazine had been injected.

constriction was then often found to be diminished initially, to increase slowly to the size found before the injection or even further. After a dose of 0.1—0.5 mg/kg the sympathetic was then found to give no secretion and no dilatation but a clear constriction for one or two hours. After that period the secretory response returned slowly, the dilator effect increasing simultaneously.

Fig. 1 shows the result of a typical experiment. Sympathetic stimulation before chlorpromazine caused a secretion, decreasing in rate in the characteristic manner. The constrictor action, likewise, diminished and was at the end of the stimulation period succeeded by a marked dilation. 15 minutes after the intravenous injection of 0.3 mg/kg of chlorpromazine sympathetic stimulation caused no secretion at all. There was scarcely any dilator response. The constriction obtained was very pronounced. It may be added that in this special case atropine had been given at the beginning of the experiment in a dose which completely abolished the secretory effect of chorda stimulation. This atropine dose had not changed the effects of sympathetic stimulation.

Chlorpromazine has by some authors been assumed to possess a ganglionic blocking action. The effects so far described here could not be due to such an effect, for postganglionic stimulation of the sympathetic nerve was affected in the same way as stimulation of the trunk in the neck. The secretory effects of adrenaline and noradrenaline were, likewise, abolished by chlorpromazine in the ordinary doses.

The secretory and dilator responses to chorda stimulation were not at all changed by the chlorpromazine doses used. This was usually true even for doses as big as 10 mg/kg. With 15—20 mg/kg the secretory effect of the chorda was diminished but usually far from abolished. In order to abolish the secretion the drug had to be given intraarterially in a dose of 1—2 mg. This effect was probably not ganglionic, for the secretory action of acetylcholine, given intraarterially, was similarly affected. The dilatation produced by stimulation of the chorda or injection of acetylcholine was even more resistant than the secretion. Only the biggest doses tolerated could reduce, but not abolish the dilator response. In the experiment of fig. 2 some of these effects of big doses of chlorpromazine are illustrated.

Chlorpromazine was found to have an effect of its own on the blood vessels of the gland. After the intravenous injection of doses big enough to abolish the sympathetic secretion there was usually an increase in blood flow through the gland for some minutes. This was the case even if the gland had been denervated at the beginning of the experiment. A vasodilatation was probably produced in other parts of the vascular bed as well, for there was usually an evanescent fall in blood pressure after the injection. With big doses given intravenously, or after close arterial injection, the vasodilatation in the gland could be as big as that produced by chorda stimulation, but even then it lasted for a few minutes only.

The effect of chlorpromazine on the rise in blood pressure usually obtained on stimulation of the sympathetic trunk varied in different experiments. Sometimes there was no pressor effect perceptible after chlorpromazine even if the constrictor effect in the gland was marked, but in other cases the blood pressure response was only slightly, or not at all affected.

Priscol (benzylimidazoline). Using this sympathicolytic agent it proved possible to separate the effects on the gland of sympathetic stimulation in a direction opposite to that brought about by

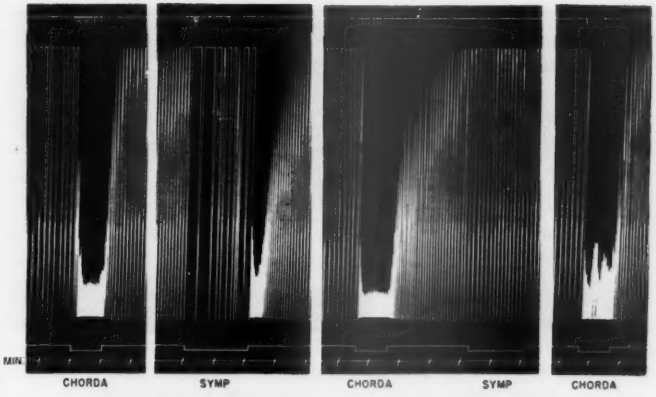

Fig. 2. Cat, 3.1 kg. Records as in fig. 1. Between the second and the third sections of the tracing injection of 10 mg/kg of chlorpromazine (20 min. prior to the chorda stimulation), between the last two sections 20 mg/kg (10 min. before chorda stimulation).

chlorpromazine, although perhaps not quite as selectively. The suitable dose was found to be about 5 mg/kg.

This dose produced a fall in blood pressure and a vasodilatation even in the denervated gland, lasting for about 5 minutes. When these effects had worn off it could be shown that the sympathetic vasoconstrictor effect in the gland had been completely abolished or strongly reduced. The secretory response, on the other hand, was retained. In some cases it was a little decreased, in others it was slightly augmented, possibly as a consequence of the abolished vasoconstriction. A pronounced vasodilatation was obtained on sympathetic stimulation after Priscol. A typical feature was, that the dilatation could now be observed earlier in the stimulation period but not until after some seconds of secretion even if there was no perceptible constriction. Further, the characteristic period of vasodilatation following cessation of the stimulation was absent after Priscol injection, or very small.

The effect of Priscol here described was pronounced for about an hour, but even after three hours the original picture of sympathetic stimulation was not quite restored. Neither secretion nor vasodilatation elicited by chorda stimulation were affected by the dose of Priscol used.

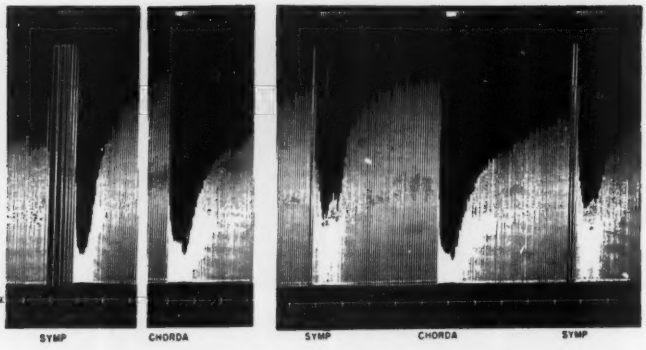

Fig. 3. Cat, 3.6 kg. Records as earlier. 10 minutes before section three of the tracing starts, Priscol 5 mg/kg had been given intravenously.

The effects of Priscol mentioned can be studied in fig. 3. Another typical experiment with Priscol is shown in a previous paper (EMMELIN 1955, fig. 2). In that experiment it can be seen that the blood pressure rise elicited by sympathetic stimulation was very little reduced by Priscol although the constrictor effect in the gland was considerably diminished.

Regitin (N-m-oxyphenyl-N-p-tolyl-amino-methyl-imidazoline). A dose of 0.5 mg/kg intravenously was found to diminish the secretory effect of sympathetic stimulation. The vasodilatation was, likewise, reduced, but the constriction very little affected. After double the dose secretion and dilatation were almost or wholly abolished; the constriction could be obtained, but markedly reduced. The blood pressure response to sympathetic stimulation was diminished. The effects of chorda stimulation on the gland were not influenced even by doses of 5 mg/kg. This dose did not increase the flow of blood through the gland acutely denervated; a small reduction could instead be observed, probably due to a fall in blood pressure.

In one case an observation, presumably exceptional, was made. The constrictor effect of sympathetic stimulation had in this cat been noticed to be feeble. After the injection of 5 mg/kg of Regitin it was absent, and the secretion as well. Instead a vasodilatation of special type was obtained on sympathetic stimulation, starting two or three seconds after the beginning of the stimulation, and increasing the flow of blood through the gland 3 or 4 times for

a period of about twenty seconds. The flow then returned to its original level in spite of continued stimulation. This dilator action of sympathetic stimulation was not affected by a dose of atropine which abolished the secretory effect of chorda stimulation. A similar, although smaller effect was possibly obtained in another experiment, in which chlorpromazine was given, but these were the only cases out of about 30 cats.

Antistin (N-Benzylanilinomethyl-2-imidazoline). This antihistamine agent has been found to have some sympathicolytic effect (see MEIER and BEIN 1950). 5 mg/kg of this drug given intravenously was found to reduce secretory, vasoconstrictor and vasodilator actions of sympathetic stimulation. 10 mg/kg had a bigger effect, but none of the actions was completely abolished. The effects of chorda stimulation were not affected, and the drug had no vasodilator action of its own in the gland.

Discussion.

The experiments show that using sympathicolytic agents it is possible to separate secretory and vasoconstrictor effects of sympathetic stimulation in the submaxillary gland of the cat. One would perhaps expect the secretory effect to be more rapidly abolished than the vasomotor effect, by analogy from the chorda tympani and atropine and considering the possibility that *constriction* is due to sympathin released in close contact with the effector cells but *secretion* to the mediator diffusing from the endings of the constrictor fibres to the glandular cells. However, one sympathicolytic agent, Priscol, is found to interfere with the constriction, another, chlorpromazine, with the secretion, whereas others such as dihydroergotamine seem to affect both responses to the same extent. It is interesting to observe that out of three imidazoline derivatives Priscol exerts its greatest effect on the constriction, Regitin on the secretion and Antistine influences the two effects to the same extent.

A striking finding is that the vasodilation, contrary to the constriction, is closely linked to the secretion and cannot be separated from it. Chlorpromazine abolishes both secretion and dilatation. In this respect it differs from atropine which abolishes the secretion but not the vasodilatation elicited by chorda stimulation. When the secretion is only reduced, by means of a

small dose of chlorpromazine, the same is the case with the dilatation. When the effect of a big dose of chlorpromazine is wearing off, the two effects return concomitantly. Priscol, on the other hand, does not only spare the secretion but the dilatation as well. When, after Priscol injection, no vasoconstriction interferes with the sympathetic dilatation, there is a marked dilatation already during the period of stimulation but not the ordinary dilatation following the stimulation period. In an exceptional cat, in which sympathetic stimulation did not evoke any secretion, vasoconstriction was the only vasomotor effect obtained. All these findings seem to support the conception of BARCROFT (1907, 1912), according to which the sympathetic vasodilatation is due to metabolites. The constriction usually obtained on sympathetic stimulation favours the accumulation of metabolites produced during the secretory activity. The constriction, therefore, is most pronounced at the beginning of the stimulation period. Accumulated metabolites may then diminish the constriction or they may even cause a temporary dilatation; if so they are quickly washed away and constriction again gets the upper hand. In this way periods of constriction and dilatation may sometimes alternate. On cessation of the stimulation the metabolites, unopposed by constrictor impulses, cause a dilatation. After Priscol, however, the metabolites are able to guarantee an adequate blood supply during the stimulation period already, and there is no marked dilatation afterwards.

In one or two cases observations were made suggesting the presence in the sympathetic trunk of vasodilator fibres for the submaxillary gland. The dilatation was in these instances obtained very early in the stimulation period and came to light when both secretion and vasoconstriction had been abolished. This effect, like that produced by chorda stimulation, was not abolished by atropine.

It might be suggested that the increased flow of blood through the gland obtained as one effect of sympathetic stimulation is due to the rise in blood pressure. This possibility seems, however, to be ruled out by the fact that the biggest increase in flow is obtained when the blood pressure has returned to its original level; furthermore, a reperfusion of a big amount of blood, raising the blood pressure much more than did sympathetic stimulation caused a much smaller increase in the flow through the gland. It was, in addition, sometimes found that chlorpromazine abolished the dilatation but left the pressor effect intact.

Summary.

The effect of some sympathicolytic agents on the secretory and vascular responses to sympathetic stimulation in the submaxillary gland of the cat has been investigated. With chlorpromazine it is possible to abolish the secretory effect, leaving the constriction intact. Regitin has a similar but far less selective effect. Priscol abolishes the constriction, sparing the secretion. Dihydroergotamine and Antistin both affect the secretion and the constriction to the same extent. The vasodilatation which is obtained on sympathetic stimulation is closely connected with the secretion and very likely due to metabolites with vasodilator action.

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References.

Barcroft, J., J. Physiol. 1907. *35.* 29 P.
— and H. Piper, Ibidem 1912. *44.* 359.
Carlson, A. J. and F. C. McLean, Amer. J. Physiol. 1908. *20.* 457.
Courvoisier, S., J. Fournel, R. Durcrot, M. Kolsky and P. Koetschet, Arch. Int. Pharmacodyn. 1953. *92.* 305.
Emmelin, N., Acta Physiol. Scand. 1955. *34.* 11.
Meier, R. and H. J. Bein, Bull. Schweiz. Akad. Med. Wissensch. 1950. *6.* 209.
Pocidalo, J. J., H. P. Cathala, J. Himbert and M. Tardien, C. R. Soc. Biol. 1952. *146.* 368.
Richins, C. A. and A. Kuntz, Amer. J. Physiol. 1953. *173.* 471.

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Acetylcholine Inactivation and Acetylcholine Sensitivity in Denervated Salivary Glands.

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The theory has been advanced that the increased effect of a chemical agent on a denervated structure is due to diminished enzymatic inactivation of the agent. (References see CANNON and ROSENBLUETH 1949, BURN and ROBINSON 1952, 1953.)

It is well known that denervation causes a decrease in the cholinesterase activity in the superior cervical ganglion (BRÜCKE 1937, COUTEAUX and NACHMANSOHN 1940, COUTEAUX 1942, SAWYER and HOLLINSHEAD 1945). SCHOFIELD (1952) has reported that removal of the ciliary ganglion induces a considerable fall in the rate of destruction of acetylcholine in the iris of cat. In cardiac muscle PIROLLI (1942) observed a fall in cholinesterase activity after bilateral section of the vagi. Experiments on skeletal muscle have given less clear-cut results. A decrease in the enzyme activity has been reported by some authors, but according to others there may even be an increase in the activity (for references see AUGUSTINSSON 1948, BROOKS and MYERS 1952). The increase of the enzyme activity, calculated per g of tissue, has, however, been attributed to an atrophy of the organ studied; COUTEAUX and NACHMANSOHN (1940), for example, who observed such an increase found the activity, calculated for the whole organ to be diminished.

Attempts have been made to correlate the sensitization to acetylcholine to a decreased cholinesterase activity. MENG (1940) found, however, that in the denervated rectus muscle of the toad

a supersensitivity to acetylcholine could be detected before the enzyme activity had begun to decline. In the serratus muscle of the guinea-pig BROOKS and MYERS (1952) found no change in the total content of true cholinesterase of the muscle whereas the threshold dose of acetylcholine decreased approximately a thousand times. They concluded that this observation "rules out any general theory designating ChE change as the agent responsible for supersensitivity".

In an investigation on the problem of "paralytic secretion" MACINTOSH (1937) examined the enzymatic destruction of acetylcholine in parasympathetically denervated submaxillary glands. The enzyme activity was found to be of the same order of magnitude in normal and denervated glands. He emphasized, however, that "statistical analysis of a much larger series of determinations would be necessary to determine whether denervation of the gland has any definite influence on its esterase content". In addition he pointed out that the operation carried out, section of the chorda tympani, is a preganglionic denervation, and that degeneration of the postganglionic neuron might reduce the esterase activity.

In the present investigation the parotid gland was studied, since in this preparation both the pre- and postganglionic parasympathetic neurons are well accessible. The effect of preganglionic denervation was also studied in a series of experiments on the submaxillary gland.

Methods.

For the experiments 39 cats weighing 3—5 kg were used. The parotid glands were left intact and denervated pre- or postganglionically. The preganglionic denervations (interruption of the tympanic nerves in the bulla) and the postganglionic denervations (cutting of the auriculotemporal nerves) were carried out as earlier described (STRÖMBLAD 1955). The submaxillary glands were left intact or the right chorda tympani was cut.

Estimation of the acetylcholine sensitivity. 13—14 days after the denervations the animals were, after preliminary ether anaesthesia, anaesthetized with chloralose (70 mg/kg body weight intravenously). The parotid or submaxillary ducts were exposed and small glass cannulae were inserted into the ducts (each cannula gave 190—200 drops out of 5 cc of distilled water).

Acetylcholine was injected in doses of 0.063, 0.125, 0.25, 0.5, 1, 2, 5, 10 and 20 μg/kg body weight into a cannula in the femoral vein aud

the drops of saliva caused by the injections were counted. After the animal had been killed by air embolism the glands were immediately removed, carefully cleansed, washed in saline, dried with filterpaper and weighed. The glands were stored at — 60° C. until the enzyme estimations were carried out (1 or occasionally 2 days later).

Quantitative estimations of enzyme activity were made manometrically (AMMON 1933) using the Warburg technique. The glands were minced with scissors and a suspension was made by adding buffer (4 times weight of gland in gram). The composition of the buffer used was $NaCl = 0.15$ M, $NaHCO_3 = 0.025$ M, $MgCl_2 = 0.04$ M (NACHMANSOHN and ROTHENBERG 1945.) The suspension was ground in a glass homogenizer at 0° C. and centrifuged at 3000 r./min. for 5 minutes. The clear supernatant fluid was then brought into the side bulbs of conical manometric flasks (0.3 cc in each). Into the main chamber of the flasks 1.7 cc of substrate-buffer solution was poured. The final substrate concentration in reaction mixture (total volume 2.00 cc) was 3.30×10^{-3} M (0.06 %) acetylcholine chloride or 2.85×10^{-3} M (0.06 %) butyrylcholine chloride.

The flasks were filled with nitrogen (95 %) and carbon dioxide (5 %) and incubated at 37° C. Readings were taken every 6 minute for 42—60 minutes starting 3 minutes after tipping. In all experiments a thermobarometer and a "substrate blank" were used and corrections for changes in these flasks were made. The carbon dioxide evolution was plotted on a graph against the time and the reading for 30 minutes was calculated and corrected for non-enzymatic hydrolysis of the substrate.

All estimations were made in duplicate.

The value thus obtained was expressed as μl CO_2 evolved/g of tissue/30 min. = b_{30} (AUGUSTINSSON 1948).

The acetylcholine concentration, 3.30×10^{-3}, was chosen since the peak of the activity-substrate concentration curves (AUGUSTINSSON 1948) for all types of glands was found at this concentration. In some experiments a small volume of eserine salicylate giving a reaction mixture molarity of 3.63×10^{-6} (AUGUSTINSSON 1948) was poured into a side bulb and tipped into the main chamber 24 minutes after estimation had begun. This was found to abolish the evolution of CO_2 almost completely and thus it could be concluded, that the reaction studied really was enzymatic breakdown of acetylcholine.

Results.

Type of Cholinesterase.

In the present work the question is investigated whether the heightened response to acetylcholine in salivary glands after parasympathetic denervation (for references see EMMELIN 1952, STRÖMBLAD 1955) can be explained by a lessened rate of enzymatic destruction of acetylcholine in the denervated gland as compared with the normal gland.

Table I.

Substrate: butyrylcholine.

Parotid glands:

Normal parotid glands:

Cat. no.	b_{30}		Weight of glands in g		μl CO_2/30 min./ gland = total amount	
	right	left	right	left	right	left
1	33	50	0.992	1.160	33	58
9	50	50	0.938	1.124	47	56
10	167	200	0.913	0.940	152	188
11	133	100	1.090	0.989	145	99
12	100	167	0.705	0.647	71	108
14	383	300	0.648	0.588	248	176
15	150	150	1.118	0.956	168	143
16	217	133	0.716	0.822	155	109
17	167	167	0.741	0.830	124	139
Mean	156	146	0.873	0.895	127	120

Right parotid gland preganglionically denervated:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./ gland = total amount	
	denervated right	normal left	denervated right	normal left	denervated right	normal left
2	150	133	1.040	1.020	156	136
5	67	183	1.060	1.081	71	198
6	133	150	0.873	0.940	116	141
7	50	50	1.188	1.274	59	64
23	400	217	0.546	0.723	218	157
Mean	160	147	0.941	1.008	124	139

Left parotid gland postganglionically denervated:

Cat. no.	b_{30}		Weight of glands in g		μl CO_2/30 min./ gland = total amount	
	denervated right	normal left	denervated right	normal left	denervated right	normal left
3	183	167	0.722	0.656	132	110
4	100	83	1.125	0.883	113	73
Mean	142	125	0.924	0.770	123	92

Table I. (continued)

Substrate: butyrylcholine.

Submaxillary glands:

Normal submaxillary glands:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./ gland = total amount	
	right	left	right	left	right	left
21	117	117	1.551	1.425	181	167
22	267	267	1.468	1.560	392	417
26	267	225	1.070	1.005	286	226
27	225	317	1.297	1.250	292	396
Mean	219	232	1.347	1.310	288	302

Right submaxillary gland preganglionically denervated:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./ gland = total amount	
	denervated right	normal left	denervated right	normal left	denervated right	normal left
23	267	267	0.772	1.263	206	337
24	100	133	0.544	0.890	54	134
Mean	184	200	0.658	1.077	130	261

For this reason acetylcholine breakdown was studied and only slight interest was paid to the type of cholinesterase responsible for the destruction. In 22 experiments, however, butyrylcholine was used as substrate. The results of these experiments with butyrylcholine as substrate are summarized in table 1. If the figures obtained here are compared with those of the experiments using acetylcholine as substrate, it is apparent that butyrylcholine is broken down by the enzyme of the salivary glands at a much lower rate than acetylcholine. Butyrylcholine is slowly, if at all, affected by true cholinesterase whereas pseudo cholinesterase attacks butyrylcholine more readily than acetylcholine (see AUGUSTINSSON and NACHMANSOHN 1949). Thus it may be concluded that the cholinesterase in the salivary glands of the cat is mainly true cholinesterase.

Table II.

Table II.

Normal parotid glands: *Substrate: acetylcholine.*

Cat no.	b_{30}		Weight of glands in g		µl/30 min./ glands = total amount		b_{30} right in per cent of b_{30} left	Total amount right in per cent of total amount left	b_{30} left in per cent of b_{30} right	Total amount left in per cent of total amount right
	right	left	right	left	right	left				
1.....	225	225	0.992	1.160	223	261	100.0	85.4	100.0	117.0
8.....	367	467	1.318	1.310	484	612	78.6	79.1	127.2	126.4
9.....	367	317	0.938	1.124	344	356	115.8	96.6	86.4	103.5
10.....	733	850	0.913	0.940	669	799	86.2	83.7	116.0	119.4
11.....	450	433	1.090	0.989	491	428	103.9	114.7	96.2	87.2
12.....	583	583	0.705	0.647	411	377	100.0	109.0	100.0	91.7
13.....	550	600	0.914	1.096	503	658	91.7	76.4	109.1	130.8
14.....	1100	1100	0.648	0.588	713	647	100.0	110.2	100.0	90.7
15.....	667	650	1.118	0.956	746	621	102.6	120.1	97.5	83.2
16.....	850	750	0.716	0.822	609	617	113.3	98.7	88.2	101.3
17.....	783	867	0.741	0.830	580	720	90.3	80.6	110.7	124.1
Mean.....	607	622 ± 78.0	0.918	0.951	525	554	98.4 ± 3.34	95.9 ± 4.75	102.8 ± 3.63	106.8 ± 5.21

Table II. (continued)

Substrate: acetylcholine.

Normal submaxillary glands:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./gland = total amount		b_{30} right in per cent of b_{30} left	Total amount right in per cent of total amount left
	right	left	right	left	right	left		
21.....	467	467	1.551	1.425	724	665	100.0	108.9
22.....	517	517	1.468	1.560	759	807	100.0	94.1
26.....	850	917	1.070	1.005	910	922	92.7	98.7
27.....	817	767	1.297	1.250	1060	959	106.5	110.5
29.....	800	783	0.980	0.985	784	771	102.2	101.7
30.....	700	700	1.083	1.086	758	760	100.0	99.7
31.....	1017	967	0.780	0.906	793	876	105.2	90.5
Mean.....	738	731 ± 70.8	1.176	1.174	827	823	100.9 ± 1.70	100.6 ± 2.75

Table III.

Substrate: acetylcholine.

Right parotid gland preganglionically denervated:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./gland = total amount		b_{30} right in per cent of b_{30} left	Total amount right in per cent of total amount left
	denervated right	normal left	denervated right	normal left	denervated right	normal left		
2	417	400	1.040	1.020	434	408	104.3	106.4
5	433	683	1.060	1.081	459	738	63.4	62.2
6	483	600	0.873	0.940	422	564	80.5	74.8
7	283	667	1.188	1.274	336	850	42.4	39.5
19	300	500	0.440	0.546	132	273	60.0	48.4
23	817	733	0.546	0.723	446	530	111.5	84.2
32	867	867	0.675	0.901	585	781	100.0	74.9
33	300	300	1.022	1.411	307	423	100.0	72.6
Mean	488	594	0.856	0.987	390	571	82.8 $\pm$8.88	70.4 $\pm$ 7.36

Acetylcholine Destruction.

Normal glands. The rate of acetylcholine breakdown was studied in 18 pairs of normal, unoperated glands (11 pairs of parotids, 7 pairs of submaxillaries). The values obtained are shown in table II. As can be seen in the table there are considerable differences between the various pairs of parotids as well as between the various pairs of submaxillaries. The two values for the glands constituting a pair are, however, strikingly similar. Thus the mean value of the right gland in per cent of the left gland using the b_{30} values is for the parotids 98.4 ± 3.34 and the corresponding value for the submaxillaries is 100.9 ± 1.70. If the values for the total content of enzyme in the glands are considered then the value for the parotid pairs is 95.9 ± 4.75 and for the submaxillary pairs 100.6 ± 2.75. The mean b_{30} value for left parotids is 622 ± 78.0 and for left submaxillaries is 731 ± 70.8. The concentration of the enzyme is thus of the same order of magnitude in the two types of glands.

Denervated glands. In 8 cats the preganglionic parasympathetic neuron to the parotid gland was destroyed on the right side whereas the left side served as a control. This method of direct comparison between the two glands of the same cat was thought preferable

Table IV.

Substrate: acetylcholine.

Right submaxillary gland preganglionically denervated: ,

Cat no.	b_{30}		Weight of glands in g		µl CO_2/30 min./gland = total amount		b_{30} right in per cent of b_{30} left	Total amount right in per cent of total amount left
	denervated right	normal left	denervated right	normal left	denervated right	normal left		
18	433	433	1.605	1.810	695	784	100.0	88.6
19	750	683	0.737	1.495	553	1021	109.8	54.2
20	833	533	0.732	1.232	610	657	156.3	92.8
23	967	1050	0.772	1.263	747	1326	92.1	56.3
24	533	433	0.544	0.890	290	385	123.1	75.3
25	550	750	1.436	1.728	790	1296	73.3	61.0
28	733	683	0.987	1.257	723	859	107.3	84.2
Mean	686	652	0.973	1.382	630	904	108.8 ± 9.86	73.2 ± 6.06

since there is as earlier pointed out considerable differences between various cats whereas the two glands constituting a pair of glands are very similar. From the figures obtained in this type of experiment (table III) it can be concluded that preganglionic denervation of the parotid gland causes a fall in the amount of acetylcholine splitting enzyme of the gland both when calculated as concentration (activity/gram; b_{30}) and for the total amount in the gland. The mean value of the denervated gland in per cent of the normal, innervated gland is for b_{30} 82.8 ± 8.88 and the corresponding value for total amount is 70.4 ± 7.36. The difference between the value, 70.4 ± 7.36, and the value for the total amount in normals, 95.9 ± 4.75, is significant ($P < 0.01$).

The right submaxillary gland was preganglionically denervated in 7 cases, the left gland serving as control. The figures arrived at in the enzyme estimations are given in table IV. The b_{30} values was in 4 cases greater for the denervated glands than for their normally innervated fellow glands, whereas the reverse was true in two cases; in the remaining case they were equal. The mean b_{30} value of the right denervated gland in per cent of the normal left gland was 108.8 ± 9.68. Thus no decrease in concentration of enzyme after denervation was found. In the case of submaxillaries, however, the weight loss was after denervation considerable and

Table V.

Substrate: acetylcholine.

Left parotid gland postganglionically denervated:

Cat no.	b_{30}		Weight of glands in g		μl CO_2/30 min./gland = total amount		b_{30} left in per cent of b_{30} right	Total amount left in per cent of total amount right
	normal right	denervated left	normal right	denervated left	normal right	denervated left		
3	617	333	0.722	0.656	445	218	54.0	49.0
4	683	417	1.125	0.883	768	368	61.1	47.9
28	1050	567	0.722	0.614	758	348	54.0	45.9
34	383	217	1.317	1.200	504	260	56.7	51.6
35	700	300	0.960	0.916	672	275	42.9	40.9
36	400	217	0.906	0.902	362	196	54.3	54.1
37	733	300	0.978	0.927	717	278	40.9	38.8
38	950	350	0.735	0.640	698	224	36.8	32.1
39	1017	367	0.925	0.730	941	268	36.1	28.5
Mean	726	341	0.932	0.830	652	271	48.5 $\pm$ 3.12	43.2 $\pm$ 2.93

the total amount of enzyme in the denervated gland in per cent of normal gland was 73.2 ± 6.06. The difference from the normal, 100.6 ± 2.75, is significant ($P < 0.01$). The figures for the total loss of enzyme are about the same in preganglionically denervated parotids and submaxillaries (70.4 and 73.2, respectively).

The postganglionic denervations made on the parotid glands affected (compare table V) the rate of acetylcholine destruction in the glands much more than the preganglionic denervations. The mean value for the postganglionically denervated gland (b_{30} values) in per cent of the normal gland was thus 48.5 ± 3.12 and the corresponding figure for the total amount in the gland was 43.2 ± 2.93. Both values differ significantly from those of normal glands. ($P < 0.001$). The differences from the preganglionic values are also significant ($P < 0.01$).

Relation Between Decrease in Enzyme Activity and Sensitization.

From the results given in the former section it was concluded that both pre- and postganglionic denervation of the parotid gland of the cat cause a fall in the activity of the acetylcholine splitting enzymes, postganglionic denervation causing a more

pronounced fall than preganglionic denervation. In an earlier work (STRÖMBLAD 1955) it was shown that pre- and postganglionic denervation cause a sensitization to acetylcholine in the gland, postganglionic denervation causing a greater sensitization than preganglionic denervation. Thus a greater fall in cholinesterase activity corresponds to a greater sensitization; this might be taken as an argument in favour of the theory that the sensitization is caused by the decrease in the enzyme activity. BURN and ROBINSON (1952, 1953), studying the effect of denervation of the nictitating membrane on its amine oxidase content and its sensitivity to adrenaline and noradrenaline, found further support for this theory. In individual cases they observed that the greater the reduction in enzyme activity the bigger the sensitivity. This correlation was demonstrated in diagrams. The enzyme content of the membrane, after excision of the superior cervical ganglion, in per cent of the normal was plotted on the abscissa and the logarithm for the degree of sensitization on the ordinate.

An attempt was made to find a similar relation between sensitivity and enzyme activity in the present experiments. Comparisons between the two glands are possible since the acetylcholine sensitivity, just as the cholinesterase activity, is practically the same in the two intact glands of the same cat; this is true both for parotid (STRÖMBLAD 1955) and submaxillary glands (EMMELIN and MUREN 1951).

The degree of sensitization was expressed as number of drops from denervated gland divided with the number of drops from the normal gland for a certain dose of acetylcholine injected intravenously (STRÖMBLAD 1955). The results of the experiments on the sensitization are given in table VI. The degree of sensitization is less the bigger the dose (for references see: CANNON and ROSENBLUETH 1949). For this reason it was thought necessary to construct diagrams both for a small dose (2 μg/kg) of acetylcholine and for a relatively big dose (10 μg/kg). Diagrams were constructed for the parotids both for the concentration and for the total amount of enzyme in the glands; for the submaxillaries only the total amount value was used for the diagrams, because the b_{30} values (the concentration of enzyme) for these glands are not reduced and it is thus apparent that no positive correlation may exist for these values. The diagrams constructed are shown in figures 1—10. The regression lines were calculated and are drawn in the figures.

Table VI.

Cat no.	2 μg/kg body weight: Number of drops, denervated right	2 μg/kg body weight: Number of drops, normal left	2 μg/kg body weight: denervated / normal	2 μg/kg body weight: log denervated / normal	10 μg/kg body weight: Number of drops, denervated right	10 μg/kg body weight: Number of drops, normal left	10 μg/kg body weight: denervated / normal	10 μg/kg body weight: log denervated / normal
Preganglionically denervated parotid glands:								
2	2 3/4	2	1.375	0.1383	4 1/2	3 1/2	1.300	0.1139
5	2 2/3	2 1/3	1.067	0.0282	7 1/2	8	0.938	— 0.0278
6	3/4	3/4	1.000	0.0000	2 3/4	2 3/4	1.000	0.0000
7	1 1/2	1 1/2	1.000	0.0000	6	5	1.200	0.0792
19	2 1/3	1 1/2	1.556	0.1918	6	4 2/3	1.286	0.1091
23	1 1/2	1 1/4	1.200	0.0792	5	4 1/2	1.111	0.0457
32	2/3	1/3	1.333	0.1249	1 1/4	2/3	1.875	0.2729
33	2 3/4	2/3	4.125	0.6154	7	3	2.333	0.3680
Preganglionically denervated submaxillary glands:								
18	2 1/4	3/4	3.000	0.4771	10 2/3	8 1/2	1.255	0.0986
19	1 1/2	1/2	3.000	0.4771	4	3 1/4	1.231	0.0902
20	3 1/4	1 3/4	1.857	0.2688	8 1/4	4 1/2	1.833	0.2632
23	3 3/4	2/3	5.625	0.7501	9 1/2	4 2/3	2.000	0.3010
24	1/3	1/8	2.667	0.4260	5 1/3	3 1/3	1.600	0.2041
25	6 3/4	3/4	9.000	0.9542	18 1/2	10 1/2	1.762	0.2460
28	2 1/2	1/2	5.000	0.6990	7 1/3	2 1/2	2.933	0.4673
Postganglionically denervated parotid glands:								
3	2 5/6	5/6	3.400	0.5315	6	2 2/3	2.250	0.3522
4	2 1/4	1 1/3	1.688	0.2274	4	2 2/3	1.500	0.1761
28	1 1/3	1/2	2.667	0.4259	4	2 3/4	1.455	0.1629
34	2 1/2	1 1/4	2.000	0.3010	5 2/3	3 1/4	1.744	0.2415
35	1 1/2	2/3	2.250	0.3522	5	1 2/3	3.000	0.4771
36	3 1/2	1 3/4	1.905	0.2799	6 3/4	3 3/4	1.800	0.2553
38	1 1/3	1/2	2.667	0.4259	2 1/2	1 1/2	1.667	0.2219
39	1 3/4	1/8	14.000	1.1461	5 1/2	1	5.500	0.7404

Figures 1—6 show the diagrams constructed for the preganglionic denervations. The regression lines for 2 μg/kg and 10 μg/kg doses for the parotid (figures 1—4) as well as the line drawn for the 10 μg/kg dose for the submaxillary (figure 6) indicate a negative correlation between enzyme decrease and sensitization. The 2 μg/kg diagram for the submaxillary glands (figure 5) indicated a positive correlation though the dispersion round the regression line is rather great. Figures 7—10 show the diagrams constructed for post-

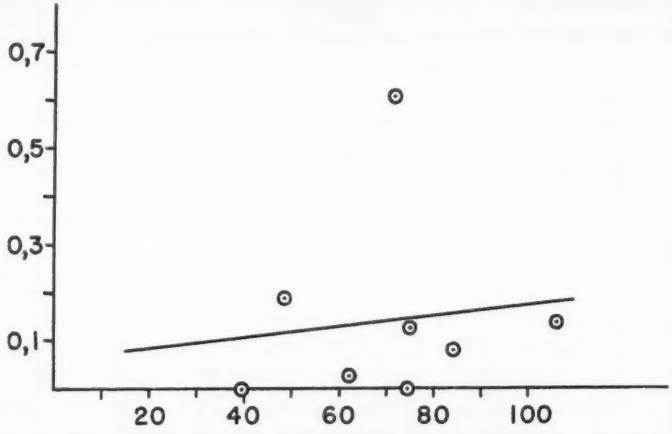

Fig. 1. Figs. 1—10 show the relation in the individual cases between sensitization to and enzymatic destruction of acetylcholine. Ordinates: Logarithm of degree of sensitization of the denervated gland in relation to the contralateral normal gland. Abscissas: Enzyme activity of denervated gland in per cent of the activity of the contralateral normal gland. Type of glands: Preganglionically denervated parotid glands. Dose used in estimating sensitization: 2 μg/kg. Enzyme activity calculated as: Total amount.

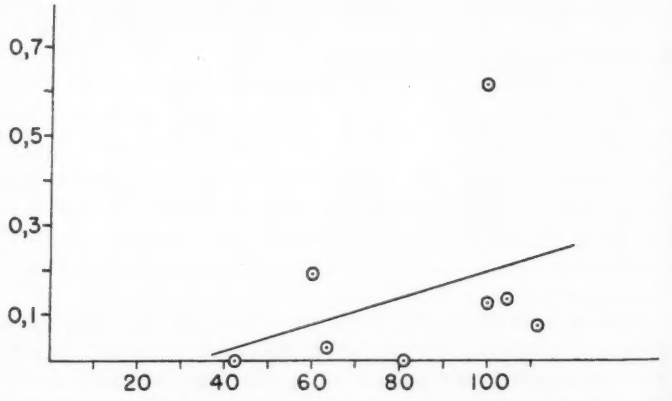

Fig. 2. Type of glands: Preganglionically denervated parotid glands. Dose used in estimating sensitization: 2 μg/kg. Enzyme activity calculated as: Concentration.

ganglionically denervated parotid glands. A positive correlation for concentration and total amount for both 2 γg/kg and 10 μg/kg

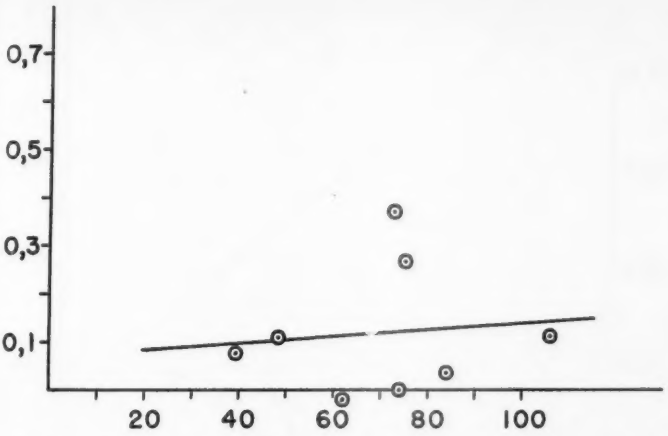

Fig. 3. Type of glands: Preganglionically denervated parotid glands. Dose used in estimating sensitization: 10 μg/kg. Enzyme activity calculated as: Total amount.

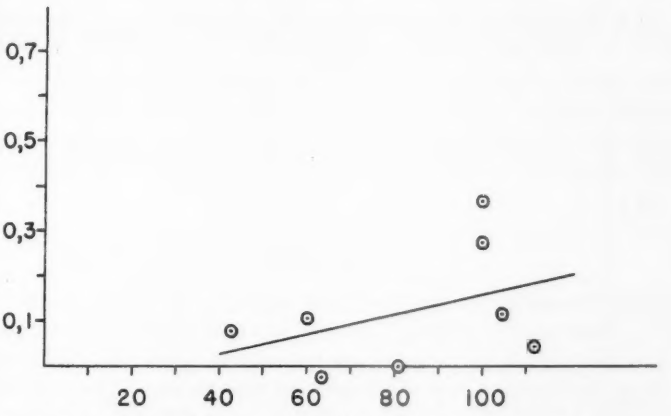

Fig. 4. Type of glands: Preganglionically denervated parotid glands. Dose used in estimating sensitization: 10 μg/kg. Enzyme activity calculated as: Concentration.

doses is indicated and furthermore the individual points are scattered rather closely round the regression line.

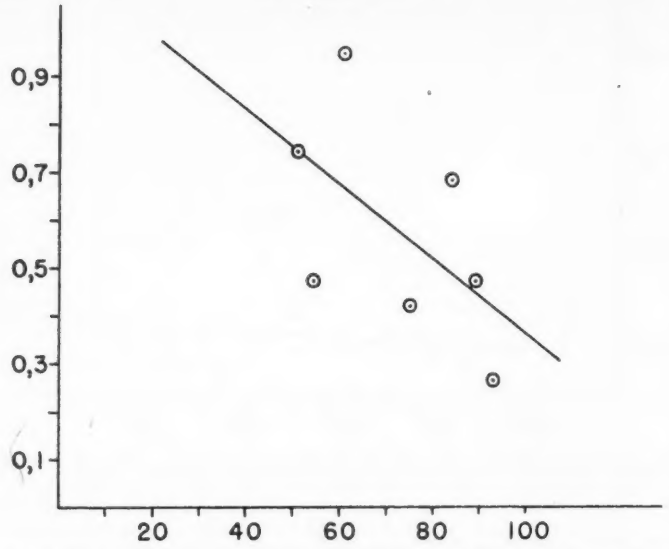

Fig. 5. Type of glands: Preganglionically denervated submaxillary glands. Dose used in estimating sensitization: 2 μg/kg. Enzyme activity calculated as: Total amount.

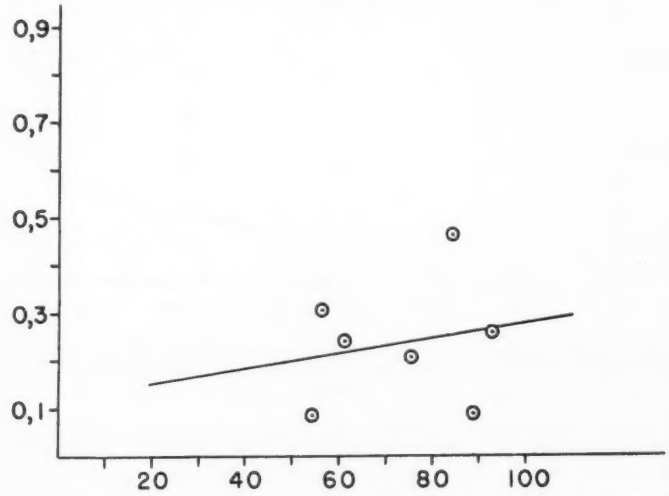

Fig. 6. Type of glands: Preganglionically denervated submaxillary glands. Dose used in estimating sensitization: 10 μg/kg. Enzyme activity calculated as: Total amount.

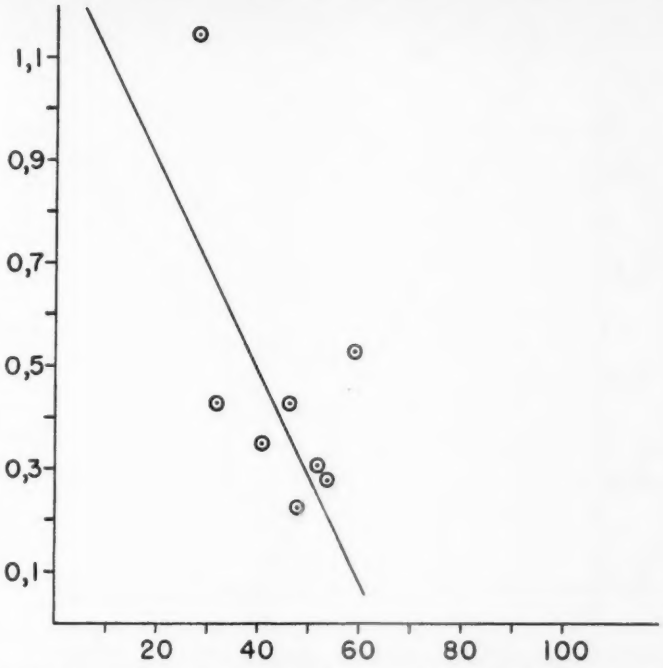

Fig. 7. Type of glands: Postganglionically denervated parotid glands. Dose used in estimating sensitization: 2 μg/kg. Enzyme activity calculated as: Total amount.

Discussion.

When examining a possible relation between acetylcholine sensitivity and enzyme activity the first problem encountered is a practical one: since denervation is followed by atrophy of the gland, should comparisons then be made using concentration of enzyme or total amount of enzyme in the gland? The weight loss of the submaxillary gland, for instance, amounts to about 30 per cent after denervation; the loss of glandular tissue might be even bigger, since there is an increase in connective tissue (RAWLINSON 1935, EMMELIN, JACOBSOHN and MUREN 1951). According to the present experiments there is no decrease in the enzyme activity of the submaxillary gland when calculated as concentration. Consequently, these results are not in accordance

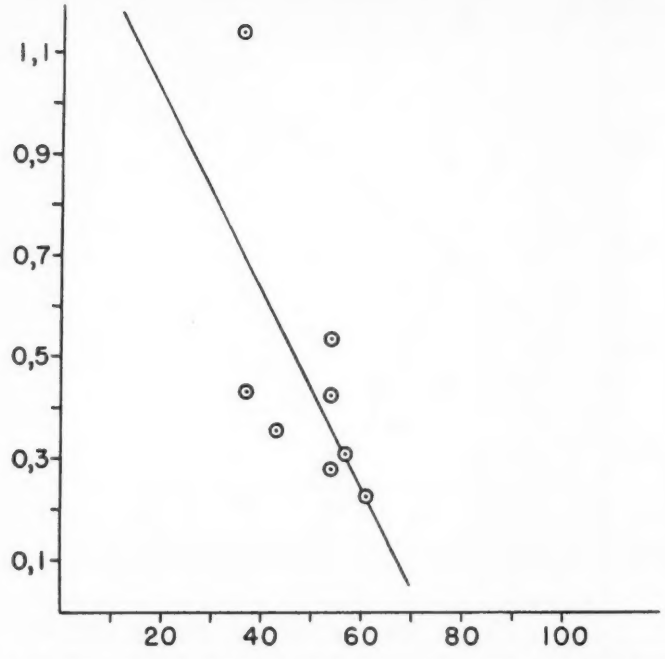

Fig. 8. Type of glands: Postganglionically denervated parotid glands. Dose used in estimating sensitization: 2 μg/kg. Enzyme activity calculated as: Concentration.

with the theory that the sensitization to acetylcholine observed is due to loss of splitting enzyme. In the following discussion interest has therefore been centred on the problem whether the sensitization can be related to a loss of activity, calculated for the whole gland.

The results show a decrease in total amount of enzyme following both pre- and postganglionic denervations. The analysis of the individual cases does not reveal a definite correlation between enzyme decrease and sensitization in single cases with preganglionic denervations. The diagrams constructed for postganglionically denervated glands do, however, suggest such a correlation.

The enzyme decrease after preganglionic denervation amounts to about 30 per cent and after postganglionic denervation to about 60 per cent. It is possible that a decrease of 30 per cent does

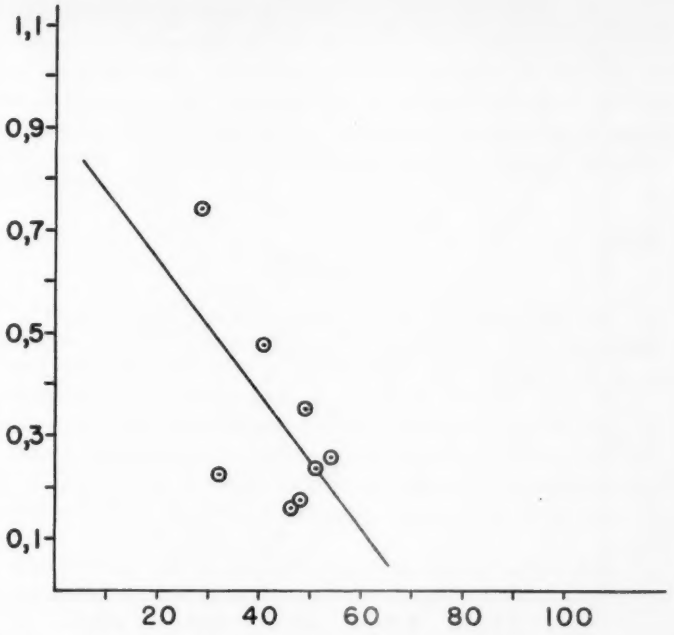

Fig. 9. Type of glands: Postganglionically denervated parotid glands. Dose used in estimating sensitization: 10 μg/kg. Enzyme activity calculated as: Total amount.

not affect the rate of acetylcholine breakdown in the gland. The results of investigations carried out with eserine (MENG 1940) and newer cholinesterase inhibitors (RIKER and WESCOE 1949, KAMIJO and KOELLE 1952, BARNES and DUFF 1953, HOLADAY, KAMIJO and KOELLE 1954) indicate that the transmission of impulses is not affected by a loss of cholinesterase of less than 50 per cent. Of especial interest is the work of RIKER and WESCOE, who made injections of DFP into the submaxillary gland and studied the effect of stimulation of the chorda tympani. These workers could find no changes in the salivary secretion in response to nerve stimulation until the total amount of acetylcholine splitting enzymes in the gland was reduced with 50—65 per cent.

In evaluating the results of experiments of the type reported here it must be borne in mind that cholinesterase is present not only in the secretory parenchyma. KOELLE (1950) has histo-

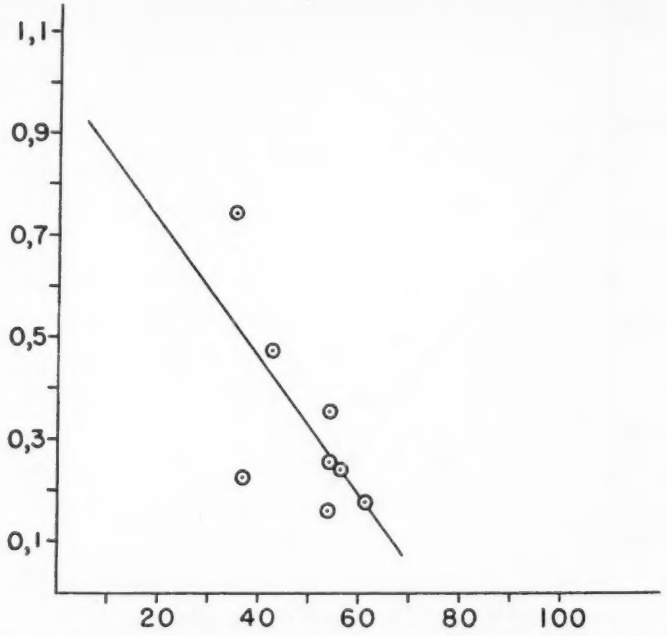

Fig. 10. Type of glands: Postganglionically denervated parotid glands. Dose used in estimating sensitization: 10 μg/kg. Enzyme activity calculated as: Concentration.

chemically shown that in the parotid gland of the cat cholinesterase may in addition be found in, for instance, the endothelial cells and circular muscle fibres of the blood vessels; in these sites true cholinesterase predominated, and in the parenchyma the acetylcholine splitting enzyme was almost exclusively true cholinesterase. The present experiments indicate that in an extract of the parotid gland the major activity is due to true cholinesterase. It cannot, however, be ruled out that the pseudo cholinesterase takes part in the process of sensitization, even if present mainly in the vascular wall, since the acetylcholine administered to test the sensitivity has to pass this wall. This consideration seems to justify an estimation of the power of the gland to destroy acetylcholine, the type of enzyme concerned not being taken into account in most experiments.

The experiments suggest that the sensitization to acetylcholine

in salivary glands observed after postganglionic denervation may be due to a reduction of the activity of the enzyme destroying the acetylcholine. The observation on preganglionically denervated glands are less conclusive; they suggest that the reduction in enzyme activity may at the most be a contributing factor in the process of sensitization. Further work is required to elucidate this problem.

Summary.

Acetylcholine destruction was studied in the normal and the denervated parotid and submaxillary gland of cat. The parotid gland was pre- and postganglionically, the submaxillary gland preganglionically denervated. In the same cats the sensitization to acetylcholine brought about by the denervations was studied.

The preganglionic denervations were found to cause a decrease by 30 per cent in the total amount of enzyme in the glands. The postganglionic denervation caused a decrease in total amount by about 60 per cent.

The possible rôle of these reductions in enzyme activity for the mechanism of the sensitization to acetylcholine is discussed.

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References.

Ammon, R., Pflüg. Arch. ges. Physiol. 1933. *233*. 486.
Augustinsson, K-B., Acta Physiol. Scand. 1948. *15*. Suppl. 52.
— and D. Nachmansohn, Science 1949. *110*. 98.
Barnes, J. Y. and J. I. Duff, Brit. J. Pharmacol. 1953. *8*. 334.
Burn, J. H. and J. Robinson, Ibidem 1952. *7*. 304.
— — J. Physiol. 1953. *120*. 224.
Brooks, V. B. and D. K. Myers, Ibidem 1952. *116*. 158.
Brücke, F. Th., J. Physiol. 1937. *89*. 429.
Cannon, W. B. and A. Rosenblueth, The supersensitivity of denervated structures, New York 1949.
Couteaux, R., Bull. biol. 1942. *76*. 14.
— and D. Nachmansohn, Proc. Soc. exp. Biol. N. Y. 1940. *43*. 177.
Emmelin, N., Physiol. Rev. 1952. *32*. 21.
— D. Jacobsohn and A. Muren, Acta Physiol. Scand. 1951. *24*. 128.
— and A. Muren, Ibidem 1951. *24*. 103.

HOLADAY, D. A., K. KAMIJO and G. B. KOELLE, J. Pharmacol. 1954. *111.* 241.
KAMIJO, K. and G. B. KOELLE, Ibidem 1952. *105.* 349.
KOELLE, G. B., Ibidem 1950. *100.* 158.
MACINTOSH, F. C., Proc. Soc. exp. Biol. N. Y. 1937. *37.* 248.
MENG, C. W., Chin. J. Physiol. 1940. *15.* 143.
NACHMANSOHN, D. and M. A. ROTHENBERG, J. Biol. Chem. 1945. *158.* 653.
PIROLLI, M., Boll. Soc. ital. Biol. sper. 1942. *17.* 438.
RAWLINSON, H. E., J. Anat., Lond. 1935. *70.* 143.
RIKER, W. F. and W. C. WESCOE, J. Pharmacol. 1949. *95.* 515.
SAWYER, C. H. and W. H. HOLLINSHEAD, J. Neurophysiol. 1945. *8.* 137.
SCHOFIELD, B. M., Brit. J. Pharmacol. 1952. *7.* 670.
STRÖMBLAD, R., Acta Physiol. Scand. 1955. *33.* 83.

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A Comparative Study of Bradykinin and Substance P.

By

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The similarity in the chemical and pharmacological characteristics of bradykinin and substance P has previously been mentioned (ROCHA E SILVA 1949, PERNOW 1953). Both substances are polypeptides with a slow contracting effect upon several smooth muscle structures and a depressing action upon the blood pressure. These effects are not influenced by atropine, antihistaminics or ganglionic blocking agents. The activity of both bradykinin and substance P is rapidly destroyed by chymotrypsin (WERLE, KEHL and KOEBKE 1951, PERNOW, to be published). However, differences have been observed concerning the chemical nature of the substances. Substance P is completely adsorbed on aluminium oxide from an alkoholic solution (PERNOW 1953), while bradykinin under the same condition is less strongly retained by aluminium oxide (PRADO, BERALDO and ROCHA E SILVA 1950). On paper chromatograms using butanol/acetic acid/water (40 : 10 : 50) as solvent the R_F-value of bradykinin on Whatman 1 paper was found to be 0.52 (ROCHA E SILVA 1951), whereas substance P on Grycksbo OB gave 0.38 (PERNOW 1953). The present paper deals with further comparative chemical and pharmacological studies of the two substances.

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Methods.

Bradykinin was prepared according to the methods described by ROCHA E SILVA, BERALDO and ROSENFELD (1949) and PRADO, BERALDO and ROCHA E SILVA (1950) by incubation of the globulin fraction of cattle blood with the venom of B o t h r o p s j a r a r a c a. The active principle was extracted with glacial acetic acid and precipitated with ether. The activity of the preparation was 8 units per mg, the standard unit being defined as the activity contained in 1 mg of Pool I (PRADO, BERALDO and ROCHA E SILVA 1950).

Substance P was obtained from cattle intestine by boiling the tissue in water at pH 4 and precipitating with ammonium sulphate according to EULER (1942). It was further purified by adsorption on aluminium oxide according to the method previously described (PERNOW 1953). The activity of the preparation was 200 Euler units per mg (EULER 1942).

Paper electrophoresis was performed in the apparatus described by KUNKEL and TISELIUS (1949). The most suitable buffer solution for separating substance P from bradykinin was found to be a 0.05 N acetate buffer (pH 4.0), using 220 V and 4 mA. After a 10 hour run the paper (Whatman No. 20) was dried at room temperature, cut in strips, and the activity tested on the guinea-pig ileum and hen rectal caecum by immersing each piece of paper in the intestinal bath. Either substance when present was rapidly eluted from the paper and gave rise to a slow contraction.

The counter-current distribution was performed in a 25 tube steel apparatus (CRAIG and POST 1949). The distribution system was *n* butanol/acetic acid/water (40 : 10 : 50), 8 ml for both phases. The experiments were performed according to the fundamental method by CRAIG and POST (1949). All lower tubes were filled with the stationary phase; fresh mobile phase in addition to the substances studied was added to tube 0. After equilibration the mobile phase was transferred to tube 1 and fresh upper phase added to tube 0. This procedure was continued until all tubes of the apparatus contained both phases. 1 ml of each lower phase was then concentrated to dryness *in vacuo* at room temperature, dissolved in distilled water and immediately tested on the guinea-pig ileum. Calculations of the theoretical curves were performed according to WILLIAMSON and CRAIG (1947).

Assay of the substances was made on isolated guinea-pig ileum, rat intestine and hen rectal caecum suspended in Tyrode's solution containing glucose. The volume of the bath was 3 ml for the guinea-pig and rat intestine and 15 ml for the hen caecum. The bath fluid was aerated with oxygen (guinea-pig and rat) or 5 per cent CO_2 in oxygen (hen caecum). The vasodepressor effects of bradykinin and substance P were studied on rabbits and cats, with the blood pressure recorded from the carotid artery.

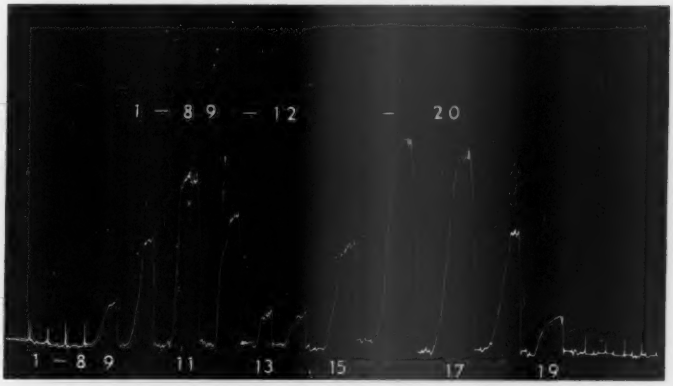

Fig. 1. Hen rectal caecum (upper tracing), guinea-pig ileum (lower tracing). Separation of bradykinin and substance P by paper electrophoresis. Each figure represents 0.5 cm of the paper from the start point to the cathode.

Results.

1. *Paper electrophoresis.* From a mixture of bradykinin and substance P of the above mentioned purity the two principles could easily be separated after electrophoresis for 8 hours at pH 4. At that pH both substances migrated to the cathode but at different speeds. As seen from figure 1 two distinctly separated peaks of activity were obtained on the guinea-pig ileum. Since only the slower moving spot gave a definite effect on the hen caecum, this was assumed to be substance P.

2. *Counter-current distribution.* In the initial experiments 15 mg of bradykinin and 7 mg of substance P were mixed and added to tube 0. After the distribution only one symmetric curve of activity was obtained with a maximum in tube 5, giving a distribution coefficient (K) of 0.30. Therefore, in the following experiments the substances were studied separately by mixing one active substance with the other, which had previously been inactivated by chymotrypsin. In so doing the dry weight of material was kept constant in all experiments. In a typical experiment 7 mg substance P was incubated with 0.1 mg crystalline chymotrypsin in Tyrode's solution (pH 8.5) at 38° C over night. After making certain that the substance was completely inactive biologically, the chymotrypsin activity was destroyed by boiling

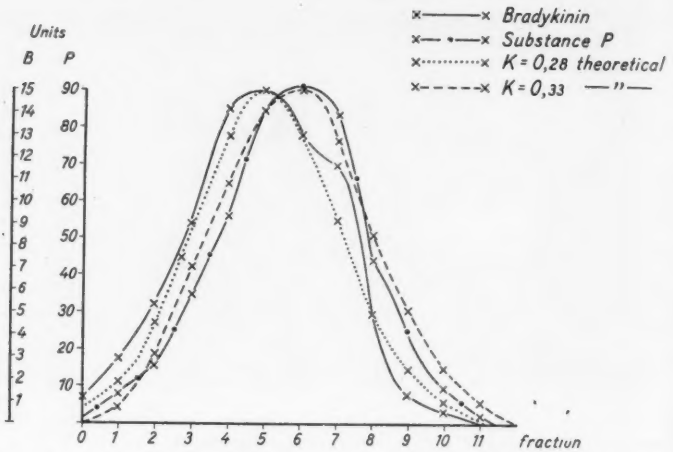

Fig. 2. Counter-current distribution of bradykinin and substance P. Solvent butanol/acetic acid/water (40 : 10 : 50).

and the whole solution concentrated to dryness *in vacuo* and again dissolved in the lower phase of tube 0. 15 mg of active bradykinin was then added to the same phase and the distribution started. After a 25 tube run the maximal yield of bradykinin activity was found in tube 4—5, giving a K of 0.28. In experiments with active substance P and inactivated bradykinin the maximal activity was obtained from tube 6—7 with a K of 0.35. Bradykinin was most stable during this procedure, giving a yield of about 70 per cent, whereas the yield of substance P was only about 40 per cent (Fig. 2).

3. *Guinea-pig ileum.* Both bradykinin and substance P gave rise to a progressively increasing tone, starting after a short latent period. The immediate contracting effect of histamine and acetylcholine was strikingly different. Bradykinin produced a more gradual increase in tone than did substance P and the relaxation after the drugs had been washed out was also slower. A time-effect relationship was obtained by measuring the time (in seconds) necessary for the successive levels of contraction to be obtained (in cms of height of contraction). In fig. 3 comparative tracings are presented and in figs. 4 and 5 the curves of contraction are plotted against the logarithm of time for both contraction and relaxation. Between reasonable limits (20—80

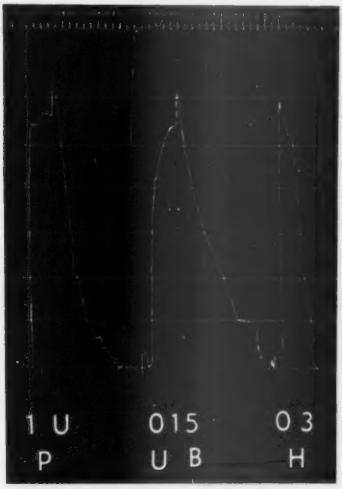

Fig. 3. Guinea-pig ileum. Bath volume 3 ml. Effect of 1 unit substance P, 0.15 unit bradykinin and 0.3 μg histamine. Time: 5 sec.

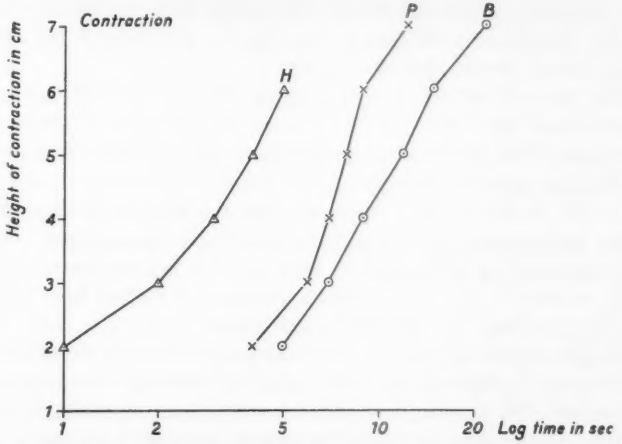

Fig. 4. Time-effect relationship of the contractions produced by histamine, substance P and bradykinin. The time is measured after application of the drugs in the bath fluid.

per cent of maximal contraction) a linear relationship between height of contraction to log-time could be observed. The curves

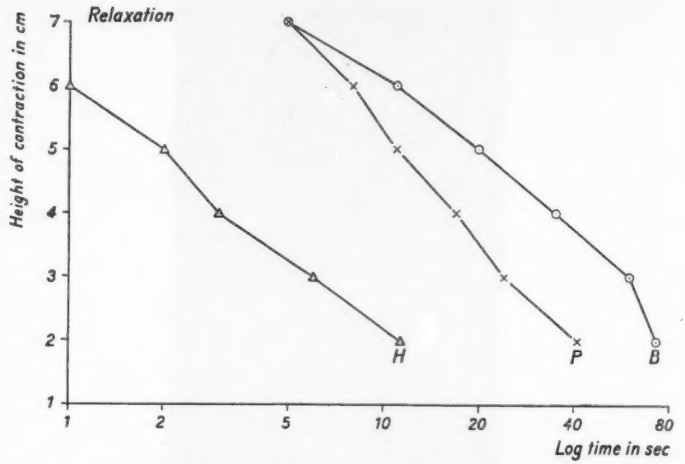

Fig. 5. The speed of relaxation after contractions produced by histamine, substance P and bradykinin. The time in sec. is measured after changing of the bath fluid.

for histamine, substance P and bradykinin were significantly different, being slower for bradykinin than for substance P and both being much slower than histamine.

The ratio of potencies of bradykinin and substance P was also determined upon the gut. 6—7 units of substance P produced the same effect as 1 unit of bradykinin, giving a ratio of activity of 6 : 1 in terms of potency.

4. *Rat duodenum and ileum* are less sensitive to both bradykinin and substance P than is the guinea-pig ileum; a slow contraction starting after a short latent period is also typical. The ratio of activity of bradykinin to substance P varied from 12 : 1 to 16 : 1 in both the duodenum and ileum.

5. *Hen rectal caecum.* The hen caecum was highly sensitive to substance P, which is in conformity with previous observations (PERNOW 1953). This preparation was, however, rather insensitive to bradykinin. A bradykinin concentration of 0.2 mg/15 ml gave rise to only a slight increase in the spontaneous movements, whereas 5 μg of substance P produced a significant increase in tone. The ratio of activity between bradykinin and substance P was lower than 1 : 20 (fig. 1 upper tracing).

6. *Rabbit blood pressure.* Both bradykinin and substance P gave

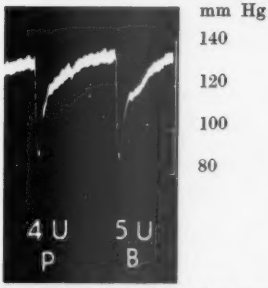

Fig. 6. Rabbit's blood pressure. Effect of 4 units of substance P and 5 units of bradykinin.

rise to a rapid and significant fall in blood pressure in the rabbit (fig. 6). 3 units of either substance produced a fall of about 20 mm Hg, giving a ratio of activity of 1. Since the bradykinin unit is 6—7 times more potent than the substance P unit on the guinea-pig ileum, substance P evidently has a much stronger depressor action than bradykinin. The return to the initial level was sometimes slower for bradykinin than for substance P.

Summary.

1. The evidence that bradykinin and substance P represent separate substances is based on counter-current, electrophoretic and pharmacological studies.

2. On paper electrophoresis bradykinin and substance P both migrate to the cathode at pH 4. Two peaks of activity are obtained due to differences in mobility.

3. Owing to different distribution coefficients between butanol and acetic acid/water bradykinin and substance P give two separate peaks of activity in the counter-current distribution.

4. Bradykinin and substance P present clear differences in pharmacological actions. The contracting effect of bradykinin in smooth muscle preparations is slower than that of substance P. The hen caecum is very sensitive to substance P but rather insensitive to bradykinin. The depressor action of substance P is more pronounced than that of bradykinin.

References.

Craig, L. C. and O. Post, Anal. Chem. 1949. *21*. 500.
Euler, U. S. v., Acta Physiol. Scand. 1942. *4*. 373.
Kunkel, H. G. and A. Tiselius, J. Gen. Physiol. 1951. *35*. 89.
Pernow, B., Acta Physiol. Scand. 1953. *29*. Suppl. 105. 1.
Prado, E. S., W. T. Beraldo and M. Rocha e Silva, Arch. Biochem. 1950. *27*. 410.
Rocha e Silva, M., Bradicinina. São Paulo 1951.
— W. T. Beraldo and G. Rosenfeld, Amer. J. Physiol. 1949. *156*. 261.
Werle, E., R. Kehl and K. Koebke, Biochem. Z. 1950. *321*. 213.
Williamson, B. and L. C. Craig, J. Biol. Chem. 1947. *168*. 687.

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Transport Form of Decanoic Acid-1-^{14}C in the Lymph During Intestinal Absorption in the Rat.

By

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Long chain saturated and unsaturated fatty acids fed as free acids, glycerides or as esters of various alcohols are almost quantitatively transported via the intestinal lymph in the ester form.

Fatty acids with 10 or less carbon atoms are, however, transported mainly via the portal blood vessels (BLOOM, CHAIKOFF and REINHARDT 1951, KIYASU, BLOOM and CHAIKOFF 1952) mainly in the free form (BORGSTRÖM 1955).

As nothing was known about the transport form of the small fraction of short chain fatty acids which is transported via the intestinal lymph (5—19 per cent of the absorbed decanoic acid according to BLOOM et al. 1951) during the absorption, this problem has been investigated with ^{14}C-decanoic acid.

Experimental.

Decanoic acid-1-^{14}C was prepared by BERGSTRÖM and PÄÄBO. The labelled decanoic acid had a specific activity of 122,000 c. p. m. per mg.

The rats were given olive oil containing 1.1 per cent free labelled decanoic acid (Table 1).

Adult male rats weighing about 250 g were used in this study. The procedure used for cannulating the thoracic duct and the treatment after the operation was that earlier described by BERGSTRÖM, BLOMSTRAND and BORGSTRÖM (1954). At least 20 hours after the operation

Table 1.

Recovery of activity in lymph fatty acids after oral administration of decanoic acid-1-^{14}C to rats. From 96 to 100 per cent of the fed activity was absorbed.

Rat no.	Per cent of absorbed activity recovered in lymph fatty acids	Per cent of lymph fat activity found in		Per cent of absorbed activity in expired CO_2
		Neutral fat	Phospho-lipids	
1	2.9	99.1	0.9	46/21 hr.
2	13.2	98.2	1.8	19/8 hr.
3	16.0	97.2	2.8	—
4	3.9	98.5	1.5	40/23 hr.

when the animals had recovered 0.5 ml of the fat mixture was fed by stomach tube. The thoracic duct lymph was then collected for 24 hours. The expired carbon dioxide was collected from some animals as earlier described (BERGSTRÖM, BLOMSTRAND and BORGSTRÖM 1954) (Table 1).

The animals were then killed and the whole intestinal tract removed in one piece and hydrolyzed together with the feces collected during the experiment and the fatty acids extracted.

The lymph fat was extracted with 20 volumes of ethanol : ether 3 : 1 and after the evaporation of this extract in vacuo the residue was extracted with light petroleum: chloroform 2 : 1. This solution was then subjected to chromatography on columns of silicic acid according to BORGSTRÖM (1952 a). Neutral lipids and eventually free fatty acids in the chloroform eluate from the silicic column were then separated using a column of Amberlite IRA-400 (OH) according to BORGSTRÖM (1952 b). No significant amount of activity was obtained as free fatty acids but all the activity could be accounted for as ester-bound decanoic acid in the lymph (Table 1).

The determination of radioactivity was done as earlier described after plating on aluminium planchets in infinitely thin layer (BERGSTRÖM, BLOMSTRAND and BORGSTRÖM 1954). Samples suspected to contain free decanoic acid were added with a drop of 0.1-n sodium hydroxide and the sodium salts of the fatty acids were directly plated on aluminium planchets.

Results and Discussion.

The results of the lymph experiments are given in Table 1. The labelled decanoic acid was absorbed almost quantitatively in 24 hours. From 3 to 16 per cent of the absorbed activity was recovered in the lymph lipids, which is in accordance with the results of BLOOM, CHAIKOFF and REINHARDT (1951).

About 2 per cent of the activity found in the lymph was in the form of phospholipids, the remainder in the form of neutral fat. Only traces of activity was obtained as free fatty acids in the lymph lipids. It is thus apparent that the transport form of short chain fatty acids in the lymph is the same as for long chain fatty acids. BORGSTRÖM (1955) has suggested that the transport way of fatty acids after absorption is determined by the extent to which the acid in question is built into the esters in the cells of the intestinal mucosa. It is interesting to note that all fatty acids transported via the lymph are incorporated to a certain degree into the lymph phospholipids. What this properly means we do not know, but it has recently been shown that there may be an interconversion of glycerides and phospholipids during the absorption process (BLOMSTRAND 1954).

With regard to the fact that free decanoic acid is found in the general circulation during the absorption (BORGSTRÖM 1955) it is interesting that this free acid is not able to pass the blood-lymph barrier and enter the thoracic duct lymph.

The very rapid appearance of large amounts of radioactivity in the expired air in the lymph cannulated rats confirm earlier observations, BLOOM et al. (1951) that decanoic acid is absorbed mainly via the portal vein and furthermore that short chain fatty acids are much more rapidly metabolized than long chain fatty acids (cf. BERGSTRÖM, BORGSTRÖM and ROTTENBERG 1952, BERGSTRÖM, BLOMSTRAND and BORGSTRÖM 1954).

Summary.

A study on the transport of decanoic acid-^{14}C in the rat via the thoracic duct has been made.

Only a minor part of the labelled decanoic acid was transported via the thoracic duct after absorption.

The major part of the ^{14}C-activity in the lymph lipids was in the form of triglycerides and the remainder as phospholipids. No significant amount of free decanoic acid could be found in the lymph.

I am greatly indebted to Professor S. BERGSTRÖM for the labelled decanoic acid placed at my disposal.

This work is part of an investigation supported by the Medical Faculty of the University of Lund.

References.

Bergström, S., R. Blomstrand and B. Borgström, Biochem. J. 1954. *58*. 600.
— B. Borgström and M. Rottenberg, Acta Physiol. Scand. 1952. *25*. 120.
Blomstrand, R., Acta Chem. Scand. 1954. *8*. 1945.
Bloom, B., I. L. Chaikoff and W. O. Reinhardt, Amer. J. Physiol. 1951. *166*. 451.
Borgström, B., Acta Physiol. Scand. 1952 a. *25*. 101.
— Ibidem 1952 b. *25*. 111.
— 1955. *34*. 71.
Kiyasu, J. Y., B. Bloom and I. L. Chaikoff, J. Biol. Chem. 1952. *199*. 415.

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Transport Form of ^{14}C-decanoic Acid in Porta and Inferior Vena Cava Blood during Absorption in the Rat.

By

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After absorption from the intestine, long chain fatty acids (C^{15}—C^{18}) are transported almost quantitatively via the lymphatic pathway. Shorter chain fatty acids, on the other hand, are recovered in the lymph from the thoracic duct of the rat in quantities decreasing with the chain length of the acid administered; thus when decanoic acid was fed the recoveries in lymph was of the order of 5—19 per cent of acid absorbed (BLOOM, CHAIKOFF and REINHARDT 1951, see also BERGSTRÖM and BORGSTRÖM). In accordance with this it has also been found that during absorption of ^{14}C-decanoic acid the ratio of fatty acid-^{14}C in the portal vein to that in the inferior vena cava was over unity (KIYASU, BLOOM and CHAIKOFF 1952).

While long chain fatty acids, fed as esters or as free acids, appear in the lymph only in the ester form, nothing was known about the transport form of the short chain fatty acids absorbed via the portal vein. This problem has now been investigated using ^{14}C-decanoic acid in combination with lipid separation procedures earlier developed (BORGSTRÖM 1952 a and b).

Experimental and Results.

Two experiments were performed. The details of one of these are given below.

An adult rat (about 250 g), starved 24 hours previous to the experiment, was fed 1 ml of olive oil containing 28.1 per cent free ^{14}C-decanoic

acid by gastric intubation.[1] The specific activity of the decanoic acid of the fed mixture was 122,000 c. p. m. per mg. One hour and a half after the feeding the rat was anesthetized with ether, the abdomen opened and blood collected simultaneously from the inferior vena cava (2 ml) and the portal vein (3 ml) into heparinized syringes. The blood samples were mixed as soon as possible with the 20-fold volumens of ethanol : ether 3 : 1 and the lipids extracted with chloroform after the evaporation of the alcohol : ether solution. The chloroform solutions of the total lipids were diluted to 50 ml. One ml each of these solutions (in duplicate) was added with one drop of 0.1-n sodium hydroxide and plated on aluminium planchets and their activity counted. Corrected for background the inferior vena cava blood lipids gave 159 c. p. m. per ml chloroform solution, corresponding to a total activity of 7,950 c. p. m. per ml of inferior vena cava blood. The corresponding figures for the porta blood lipids were: 547 c. p. m. per ml chloroform solution; total activity in 3 ml porta blood lipids 27,400 c. p. m. and 9,033 c. p. m. per ml porta blood.

The chloroform solutions containing the total lipids were then subjected to chromatography on columns of silicic acid as earlier described (BORGSTRÖM 1952 a). The chloroform eluate from the column containing neutral lipids and free fatty acids was made up to 50 ml and the activities assayed. The total activities of the neutral lipid fatty acid fraction from the inferior vena cava lipids were 4,800 c. p. m. and from the portal blood 18,800 c. p. m. both corrected for losses due to previous sampling. The neutral lipid fatty acid fractions were then passed through a column of the ion exchanger IRA-400 (OH) (BORGSTRÖM 1952 b, 1954). The neutral lipid fractions recovered from these separations contained a corrected total activity of 1,200 and 1,410 c. p. m. for the inferior vena cava and porta blood.

The activities of the phospholipids and free fatty acids were calculated as differences.

In the second experiment 4 rats were given olive oil containing 10 per cent free ^{14}-C-labelled decanoic acid (specific activity of the decanoic acid 18,000 c. p. m. per mg) and the inferior vena cava and porta blood collected $1^1/_2$ to 3 hours after the feeding. The pooled blood samples in this experiment made 11 ml each of inferior cava and porta blood and were worked up as in experiment 1.

To identify the labelled acid recovered from the IRA-400 columns, these fractions were added with 5 mg inactive decanoic acid and subjected to reversed partition chromatography using 45 per cent aqueous acetone as mobile phase and paraffin as stationary phase on chlorosilane treated kieselguhr as supporting medium (cf. HOWARD and MARTIN). The effluent was collected in 2 ml fractions which were titrated and assayed for radioactivity after evaporation on aluminium planchets.

The results of the chromatography of the free fatty acids recovered from the porta blood lipids showed that the activity was located exclusively inside the decanoic acid band.

[1] The ^{14}C-decanoic acid used was kindly supplied by Professor S. BERGSTRÖM.

Table 1.

Distribution of lipid-^{14}C between portal vein and inferior vena cava blood lipids after feeding decanoic acid-^{14}C to rats.

Experiment	Total lipid ^{14}C/ml: portal vein blood/ inferior cava blood	Conc. of free ^{14}C-decanoic acid in:		Distribution of radioactivity in lipid fractions of:					
				porta blood lipids			inferior cava blood lipids		
		portal vein	inferior cava vein	neutral fat	phospholipids	free fatty acids	neutral fat	phospholipids	free fatty acids
		mg per cent							
1.....	2.3	4.7	1.5	5.1	31.4	63.5	15.1	39.6	45.3
2.....	4.0	3.8	0.6	9.3	17.0	73.7	17.6	32.0	50.4

Results and Discussion.

The results obtained in the two experiments are summarized in table 1.

The ratio of specific activity in the porta blood lipids to that of the inferior vena cava blood lipids found: 2.3 and 4.0, are in good agreement with those reported by KIYASU, BLOOM and CHAIKOFF, *i. e.* 1.3—9.7 (mean of eleven 3.1). It is apparent that the largest part of the decanoic acid absorbed via the portal vein is transported in the form of the free acid. This is an interesting contrast to what has been found for the transport form of long chain fatty acids after absorption. These are found in the lymph from the small intestine exclusively in the ester form (cf. BERGSTRÖM and BORGSTRÖM). Also the fraction of decanoic acid that is transported via the lymphatic route has been found to be in the ester form (BLOMSTRAND). It therefore seems possible that the transport way of fatty acids after absorption is determined by the extent to which the acid in question is built into esters in the cells of the intestinal mucosa, *i. e.* by the specificity of the intracellular enzymes for fatty acids of different structure.

The concentration of the labelled decanoic acid in the portal blood during absorption in our experiments can be calculated from the specific activity of the fed decanoic acid and the activity found in the blood. Such calculations give the values of 7.4 and 5.1 mg per cent of total decanoic acid and 4.7 and 3.8 mg per cent for free decanoic acid in experiments 1 and 2.

Also in the inferior vena cava blood lipids the largest fraction of radioactivity was found in the form of the free acid even though here the concentrations were lower, *i. e.* 1.5 and 0.6 mg per cent. Apparently the free acid entering the portal blood is not completely removed from the circulation by passing the peripheral tissues and a considerable blood level is maintained in the general circulation during the absorption period.

Summary.

Decanoic acid-1-^{14}C was fed to rats and the radioactivity of different lipid fractions of the portal vein blood and the inferior vena cava blood were studied in two experiments.

The concentration of lipid-^{14}C was found to be 4.0 and 2.3 as high in the portal blood as in the inferior vena cava blood.

The largest part of the ^{14}C-activity in the portal blood as well as in the inferior cava blood was found in the form of free decanoic acid; the figures for portal blood being 63.5 and 73.7 per cent of the total activity as the free acid.

The concentration of free ^{14}C-decanoic acid in the portal blood during absorption was calculated to be 4—5 mg per cent.

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References.

Bergström, S. and B. Borgström, Progress in the Chemistry of Fat. Vol. III. Pergamon Press, London. 1955.

Bloom, B., F. L. Chaikoff and W. O. Reinhardt, Amer. J. Physiol 1951. *166*. 451.

Blomstrand, R., Acta Physiol. Scand. 1954. *30*. 231.

Borgström, B., Acta Physiol. Scand. 1952 a. *25*. 101.

— Ibidem 1952 b. *25*. 111.

— Ibidem 1954. *30*. 231.

Howard, G. A. and A. J. P. Martin, Biochem. J. 1950. *46*. 532.

Kiyasu, J., B. Bloom and I. L. Chaikoff, J. Biol. Chem. 1952. *199*. 415.

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Propagation Velocity in Electrically Activated Muscle Fibres in Man.

By

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The appearance of the action potential of the motor unit, and the development of its mechanical response, depend on the rate of conduction of the excitatory process over its fibres. In isolated frog muscle fibres, the propagation velocity of the action potential varies proportionally with the circumference of the fibre (HÅKANSSON 1954). In view of the fact that a muscle consists of fibres with largely different diameters, corresponding variations in propagation velocity might be expected, and might explain the variations in action potential duration in different points of a muscle.

In man, propagation velocities have been determined at voluntary effort (DENSLOW and HASSETT 1943). However, the interpretation of the results is complicated by the fact that the motor unit potential represents the activity of many fibres with spatially dispersed end plates. Therefore, in the present study, an attempt was made to measure the propagation velocity of the impulse over as few simultaneously activated fibres as possible. For this purpose, action potentials were recorded at different distances along the long axis of the muscle from a locally stimulated point. The study was combined with an examination of the distribution of fibre diameters in biopsies from subjects of different age.

Method.

The measurements were performed on the brachial biceps of 17 subjects aged 20—74 years, and two children, three and five years old, without signs or symptoms of neuro-muscular diseases.

Stimulation.

The stimulus consisted of rectangular voltage pulses of 60—100 μ sec. duration and 3—15 V amplitude. It was applied through an intramuscular bipolar needle electrode, the distance between the centers of the stimulating surfaces being 0.2 mm. The area of the stimulating surface was $2 \cdot 10^4$ μ^2 for each core of the electrode.

The stimulus artifact was reduced to a minimum by connecting the bipolar electrode over a screened transformer to the output terminals of the stimulator. The primary and secondary coils of the transformer were each surrounded by separate screens (S_1, S_2, Fig. 1 C). The terminals of the secondary coil were connected to the cores of the stimulating electrode (E_1, E_2), and the screen (S_2) to the cannula of the bipolar electrode (E_3). The screen (S_1) surrounding the primary coil was earthed.

The function of this circuit is illustrated by the diagrams in Fig. 1, A—C. In A, the stimulating current i_S spreads in the muscle between the stimulating electrode (E) and the earth electrode (G). The artifact (V_a) is the sum of the potential drop ($i_S \cdot G$) occurring over the earthed electrode and the potential difference (V) arising from the distribution of the stimulating current within the muscle itself. In Fig. 1 B, the connection of the stimulating current to earth is avoided by an unscreened transformer. However, the high frequency components (i_C) of the stimulating current can still reach G over the capacities C_1 and C_2. These components were eliminated by the screen around the primary coil of the transformer (S_1) shielding the current from C_1, and the screen around the secondary coil separating the current from C_2 from earth. This is the circuit used in the present study and is illustrated in Fig. 1 C. It is essential that the screen S_2 around the secondary coil is insulated from the screen around the primary coil and *connected to the muscle* via a low impedance, for example through the cannula (E_3) of the stimulating electrode.

With this arrangement the size of the stimulus artifact depended only on the current spread in the muscle directly arising from the stimulating electrode (E_1 and E_2). The size of the artifact depends then on the orientation of the recording electrodes relative to the stimulating current and is proportional with the distance between the two recording electrodes. The orientation of the recording electrodes in the current field might be difficult to control, but the artifact is essentially reduced, when leading off between electrodes with short distance as between core and cannula of a concentric electrode (Fig. 1 C). The artifact might be further reduced by leading off with bipolar electrodes where still

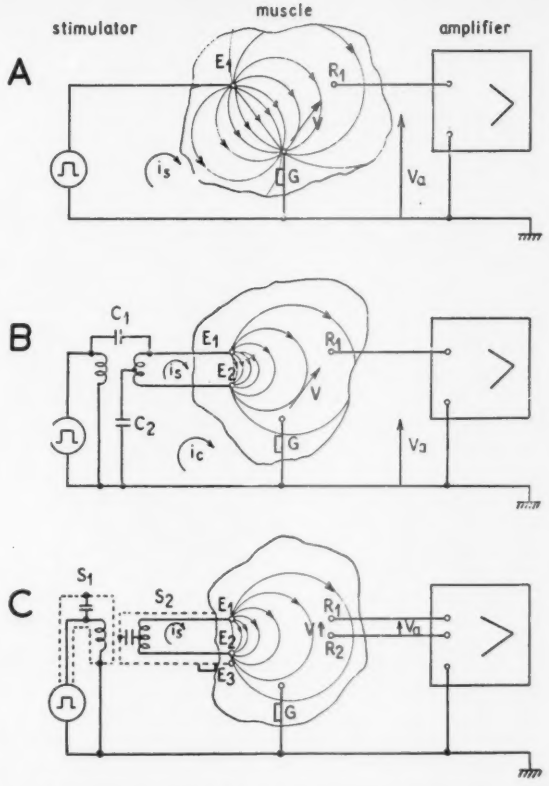

Fig. 1. Arrangement for reducing the stimulus artifact.

A. Large artifact (V_a) when stimulating current (i_S) passes through an earth electrode (G) common for stimulator and amplifier. V represents the potential difference from i_S between G and recording electrode R.

$$V_a = V + i_S \cdot G.$$

B. Artifact somewhat reduced by passing the stimulating current to a bipolar electrode (E_1, E_2) via a transformer. Only high frequency currents (i_C) pass through G and the coil capacities C_1 and C_2.

$$V_a = V + i_C \cdot G.$$

C. Artifact still more reduced by a doubly screened transformer with S_1 connected to ground and S_2 to the muscle via the low impedance E_3. Thereby the current flow through G is prevented. V is reduced by leading off with two electrodes at a short distance, *e. g.* a concentric electrode.

$$V_a = V.$$

shorter electrode distances can be obtained. However, with a distance of less than 0.5 mm between the cores of the electrode, the recorded action potential is also reduced in amplitude and this outweighs the reduction in artifact. Therefore, in the present study concentric electrodes were used for leading off. For stimulation, the bipolar electrode was found to be more suitable than the concentric. The artifact was 10—50 times greater with the concentric electrode, due to the spread of the stimulating current over a larger area when the cannula was used as one electrode. The present method of stimulation allowed recording at a distance of 10—15 mm from the stimulating electrode without distortion of the action potential by the stimulus artifact. In fact, the artifact was so much reduced that it was necessary to signal the moment of stimulation by introducing a constant signal coincident with the stimulus at that stage of the three amplifiers which follows the amplitude control (Fig. 2).

The stimulating circuit differs from the customary ones and has, therefore, been described in some detail. The doubly screened transformer with the screen around the secondary coil connected to the stimulated tissue either via a separate electrode of low impedance or via one of the stimulating electrodes has proved of value in experiments on isolated nerves and muscle fibres.

Recording.

A three channel DISA electromyograph was used for recording of evoked potentials. The concentric recording electrodes had a diameter of 0.65 mm, a core diameter of 0.1 mm, and a tip angle of 20°. Details with respect to physical properties of the differential amplifiers and the electrodes are given in a previous paper (BUCHTHAL, GULD and ROSENFALCK 1954). Care was taken to determine the distances between the recording and the stimulating tips *within* the muscle as accurate as possible, by correcting the electrode distances measured on the skin for the depth and for the angle at which the electrodes were inserted into the muscle.

The action potentials were recorded with single sweeps on photographic paper moving at a speed of 5 cm per second. The sweep was synchronized with the stimulation, the latter being delayed by an adjustable interval with respect to the start of the sweep. The sweep velocity was 1 mm per msec. (exceptionally 0.5 mm per msec.) and the frequency 2—4 per sec. A block diagram of the stimulating and recording arrangement is given in Fig. 2.

Procedure.

With the subject in a recumbent position and the arm extended, a line was drawn along the muscle to indicate the fibre direction and facilitate the placement of the different electrodes. With the stimulating electrode placed as far distal in the muscle as possible, nerve and end plate stimulation was unlikely. In the short head of the brachial

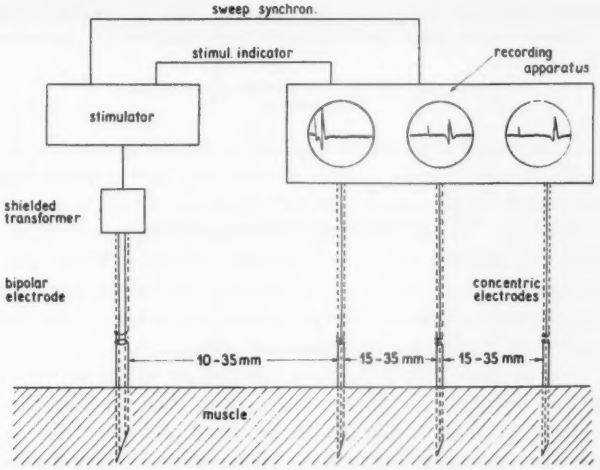

Fig. 2. Arrangement used for direct stimulation of muscle fibres in situ and for recording of the evoked potentials.

biceps, the majority of the terminal nerve endings are situated in the proximal third of the muscle (BUCHTHAL, GULD and ROSENFALCK 1955).

In addition to the bipolar stimulating electrode, at least three electrodes were used for leading off at a distance of up to 110 mm proximal to the point of stimulation. At each recording point, the action potential was first sought at suprathreshold stimulation. Then, for steps of decreasing strength of stimuli, maximum amplitude of the response was sought by small displacements of the electrode, until at threshold a small diphasic potential with a steep gradient was obtained on all electrodes. With three to five electrodes, this procedure required one to two hours. Records were taken with gradually varying stimulus intensity. Intramuscular temperature was determined by inserting a thermocouple into the muscle.

Biopsies.

For a determination of the distribution of muscle fibre circumference biopsies were examined from the brachial biceps of 19 normal subjects. After incising the skin and the fascia under local anesthesia (5 cc xylocain without adrenalin), one cc of muscle was removed, care being taken to touch only the ends of the sample with metal instruments. The sample was suspended on cork, kept in 0.9 per cent saline for two hours and fixed in four per cent neutral formalin.[1] After embedding in paraffin, 5 μ

[1] Our thanks are due to ERNA CHRISTENSEN, M. D., Laboratory of Neuropathology, Institute of Pathology, University of Copenhagen, for preparing the biopsy samples.

transverse sections were cut and stained with hematoxylin-eosin. The measurements were performed on magnifications of microphotographs.

Results.

With electrical stimulation applied to the distal portion of the brachial biceps (muscle length 15—20 cm), action potentials of the activated fibres could be picked up proximally up to a distance of at least 10—11 cm from the stimulating electrode. This was possible for all sites of the stimulating electrode. When the stimulus had a strength of 1.5—2 times threshold, the evoked action potentials were similar in duration and amplitude to the motor unit potentials recorded at voluntary effort.

If two or more electrodes were inserted along the activated fibres, the propagation velocity could be determined from the displacement in time of the action potential and the distance between the recording electrodes. However, with suprathreshold stimuli it turned out that the potential at the different electrodes did not necessarily originate from the same fibres. Moreover, the potentials led off at a larger distance from the stimulating electrodes consisted of several spikes whereby uncertainties arise as to what part of the complex potentials (column 2, Fig. 3) are correlated with the single spike recorded near the point of stimulation (column 1, Fig. 3). Therefore, the propagation velocities determined from the arrival of the action potentials initiated by superthreshold stimuli showed variations (2—10 m/sec.) exceeding considerably those which actually occur for the various fibre groups in different parts of the muscle.

That *different* fibres might give rise to the spikes recorded at the different electrodes could be demonstrated by gradually reducing the strength of the stimulus. The potential recorded at one electrode might then disappear entirely while action potentials were still recorded at one or several of the other electrodes. It was therefore necessary to confine the stimulus to as few fibres as possible and to ascertain by gradually decreasing and increasing the strength of stimulation that the threshold for the appearance of the action potentials was the same at all electrodes.

The potentials recorded at threshold stimulation were similar to those recorded from isolated frog muscle fibres in Ringer's solution (BUCHTHAL, GULD and ROSENFALCK 1954). They were mainly diphasic with a steep gradient from the positive to the negative de-

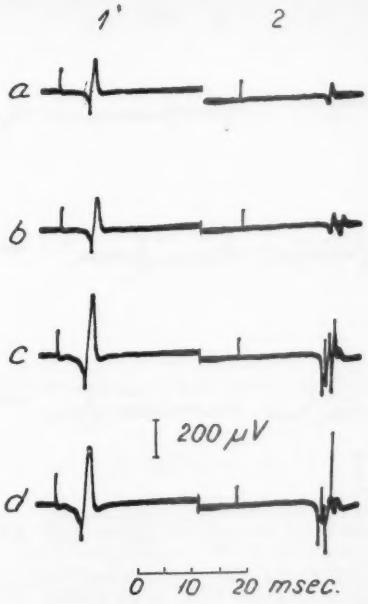

Fig. 3. Action potentials recorded simultaneously at (1) 27 and (2) 70 mm distance from the recording electrode at progressing stimulus strength from low (a) to high (d). Brachial biceps, 36.5° C.

flection (Fig. 4). The total duration was 2—4 msec. and the amplitude 20—200 μV. Duration, amplitude and shape were the same as in fibrillation potentials which are considered to represent the response of individual muscle fibres.

In recording with a special electrode containing 7 different leads at distances of 0.2 to 2 mm, it was found that the potential led off at threshold stimulation was confined to an area of approximately 1.5 mm in diameter. This is about the same as the area over which the action potential of the isolated frog's fibre can be picked up.

With a slight increase in stimulus strength, the response increased in amplitude, indicating an increase in the number of activated fibres. With further increase in stimulus strength, new spikes appeared indicating that additional groups of fibres had been activated. The new spikes may arise either from fibres with different propagation velocity or fibres with a different starting point

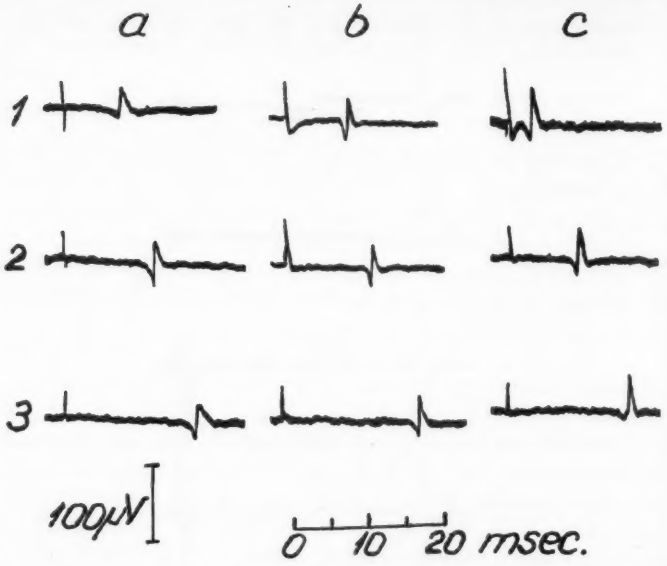

Fig. 4. Three examples for the propagation of action potentials evoked by threshold stimulation of muscle fibres in the brachial biceps (36.5° C,).

	muscle a	muscle b	muscle c
distance between recording and stimulating electrodes (in mm)			
electrode 1	34	24	11
electrode 2	48	40	37
electrode 3	68	64	61
propagation velocity (m/sec.)	3.35	4.4	4.0

for the wave of excitation. In Fig. 5, an example is given in which the new spike which appeared at increasing strength of the stimulus had a faster propagation velocity than the response to threshold stimulation (4.1 and 3.6 m/sec. respectively). This is seen from the progressive increase in the time interval between the two spikes with increasing distance from the stimulating electrode, there being no appreciable time interval between the two spikes at the electrode closest to the point of stimulation. When, on the other hand, a measurable time interval between the spikes was apparent at the recording electrode nearest to the point of stimulation, the new spikes must be attributable to a different starting point.

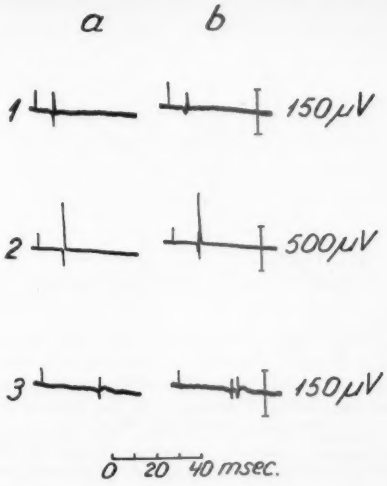

Fig. 5. Activation of groups of muscle fibres with different propagation velocities at suprathreshold (b) as compared with threshold (a) stimulus. Brachial biceps, 36.5° C. Distance between stimulating and recording electrode for electrode 1 = 33 mm, electrode 2 = 50 mm, electrode 3 = 99 mm. Propagation velocity with threshold stimulus 3.6 m/sec., with suprathreshold stimulus 3.6 and 4.1 m/sec.

For the same spike, the arrival time increased linearly with the distance of the recording from the stimulating electrode (Fig. 6). Extrapolating the curve to zero time, the starting point of the response was sometimes found to be at a considerable distance from the stimulating electrode up to 8 mm proximally or distally. The recti-linear increase in arrival time of the action potential with increasing distance from the stimulating electrode is an expression of a constant propagation velocity over the portion of the muscle investigated. Moreover, it shows that the muscle fibres pass uninterrupted over this distance and it excludes the participation of nerve fibres in the propagation.

In ten subjects the propagation velocity varied between 3.3 and 5.2 m/sec. and the mean velocity was 4.02 ± 0.13 m/sec., at an intramuscular temperature of 36.5° C. (Table 1). Different fibre groups within the same muscle had a propagation velocity not varying more than 5—15 per cent (0.2—0.5 m/sec.). The age did not cause significant variations; two subjects, 74 years old, showed propagation velocities of 4.2 and 4.4 m/sec., and two

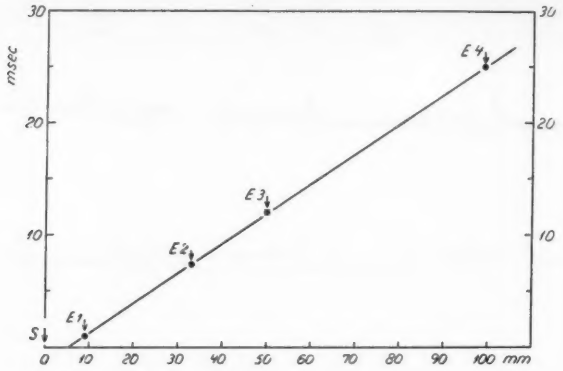

Fig. 6. Arrival time of the action potential at electrodes (E) placed at different distances from the stimulating electrode (S) in the brachial biceps of H. E. at threshold stimulation (36.5° C.).

Abscissa: distance between stimulating electrode (S) and recording electrode (E). *Ordinate:* time of arrival of the action potential, stimulus at zero time.

Table 1.

Propagation velocity in the brachial biceps at threshold stimulation in the distal portion (36.5° C.).

Subject	Age	Velocity m/sec.
P. S.	20	3.65
D. J.	21	3.35
—	—	3.80
P. N.	21	3.75
H. E.	21	4.60
H. E.	21	3.95
—	—	3.70
S. Z.	23	5.20
E. A.	23	4.40
J. S.	26	3.50
—	—	3.30
F. B.	47	3.90
—	—	4.15
E. B.	74	4.20
—	—	4.40
B. B.	74	4.40
	Mean value	4.02 ± 0.13

children, three and five years of age, had velocities within the adult range.

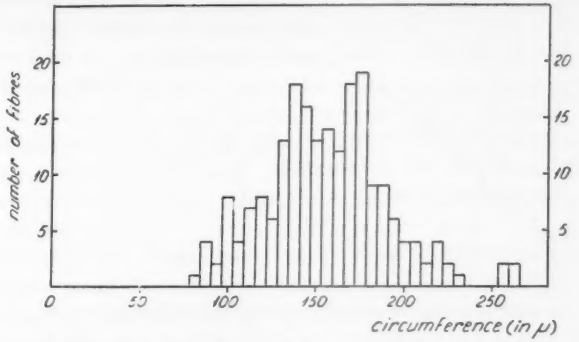

Fig. 7. Distribution of circumferences of muscle fibres in a brachial biceps (female, 62 years of age) 208 fibres. Mean circumferences = 153 μ.

Since the propagation velocity in the isolated muscle fibre increases with the fibre circumference (HÅKANSSON 1954), *fibre diameters* have been measured in biopsies of 19 normal subjects, 1—60 years of age. The muscle fibres have an elliptic cross section, with the large diameter exceeding the small by an average of 45 per cent. For fibres of the same muscle, the circumference varied 2.5—3 times (Figs. 7, 8). In muscles from subjects 1—30 years of age, the mean circumference increased approximately five times with age (Fig. 9).

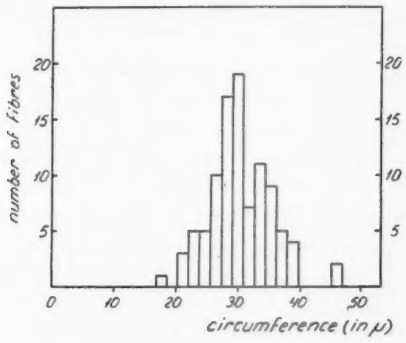

Fig. 8. Distribution of circumferences of muscle fibres in a brachial biceps (child, one-year-old) 98 fibres.

Mean circumference = 30.3 μ.
Standard deviation = 5.0 μ.

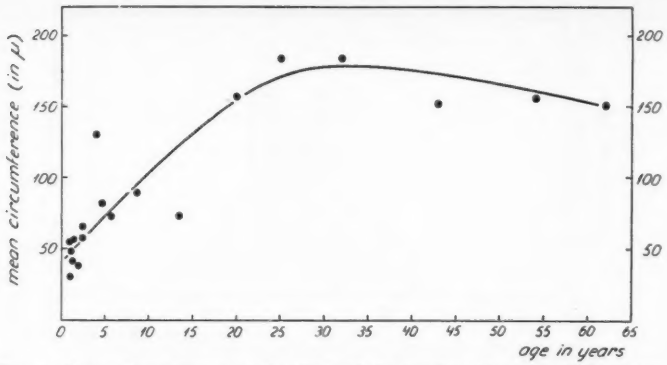

Fig. 9. Mean circumference of the fibres of the brachial biceps at different age.

Discussion.

The main result of the present study was a strikingly small variation in propagation velocity within the same muscle and in different subjects. Confining the present measurements to potentials recorded at threshold stimulation, one might object, would select only specially excitable fibres. However, the bipolar electrode used for stimulation produced a very non-homogeneous current field and the variation in threshold for the different fibres was small as compared with the changes in the field gradient over a cross-section of fibres. In other words, a fibre with a high threshold situated near the electrode had as great, or greater, chances of being activated as fibres with a lower threshold at a slightly greater distance.

It had been expected that differences in propagation velocity over the fibres might account for the spread in action potential duration obtained in different points of a muscle at voluntary effort. Assuming the same small range of propagation velocities at voluntary effort as with electrical stimulation, the question arises to what extent the spread in propagation velocity actually can explain the differences in duration, which, in the brachial biceps, may amount to 10 msec. The different propagation velocities of the fibres of a motor unit will cause an increase (Δt) in the action potential duration with increasing length (L) over which the impulse has travelled:

$$\Delta t = \frac{L}{V_1} - \frac{L}{V_n},$$

where V_l represents the minimum and V_u the maximum propagation velocity within the motor unit.

With L in the brachial biceps maximally = 8 cm and with the maximal variation for the propagation velocity in the same muscle ($V_l = 3.7$ m/sec., $V_u = 4.3$ m/sec.), the increase in duration due to propagation is maximally 3 msec. Hence, the major part of the actually occurring 10 msec. variation in the duration of the different action potentials within the same muscle must be due to other factors.

Considering the large variation of fibre circumference in a single muscle (SCHWALBE and MAYEDA 1890 and present study) and the proportionality between circumference and propagation velocity in the isolated fibre (HÅKANSSON 1954), one should have expected a 7—10 times larger variation in propagation velocity than was actually found. This implies that more than one fibre is activated even at threshold stimulation. The action potentials of adjacent fibres with different circumference must then be synchronized and the result must be a propagation velocity with a small variation. A similar mechanism has been described for frog's nerve fibres (KATZ and SCHMITT 1940).

In spite of an increase in fibre circumference by a factor 5 between 1 and 30 years, the propagation velocity was independent of the age of the subjects. It can only be assumed that age induced changes in the properties of the muscle which compensate the effect of the increased circumference.

In the isolated frog's muscle fibre with the same circumference as the mean value of the human biceps, the propagation velocity is 1.4 m/sec. at 20° C. In the temperature range 15—20° C., the velocity increases 0.15 m/sec. per degree (HÅKANSSON 1954). Extrapolating to a temperature of 36.5° C., a velocity of 3.9 m/sec. is obtained, *i. e.* the difference in the propagation velocity between frog and human muscle can be accounted for solely by the difference in temperature.

For the cat's soleus, ECCLES and O'CONNOR (1939) found velocities between 2.9 and 4.8 m/sec. and for the peroneus tertius between 4 and 7 m/sec. They stimulated a minute nerve twig innervating a small strip of superficial muscle fibres. With supramaximal nerve stimulation JARCHO, EYZAGUIRRE, BERMAN and LILIENTHAL (1952) found in the rat's m. gracilis conduction rates from 3.4 to 5 m/sec. for the fibres of a single muscle, *i. e.* a variation three times larger than in the present experiments. The spread is,

however, still considerably smaller than expected from the large differences in diameter and indicates the presence of a mechanism of mutual synchronization also at indirect stimulation. For fibrillation potentials the propagation velocity varied in different fibres between 2.66 and 2.84 m/sec., *i. e.* lower values and a significantly smaller spread than for the electrically evoked potentials (JARCHO, BERMAN, DOWBEN and LILIENTHAL 1954), and a spread similar to that found in the present study. Provided that fibrillation potentials are not confined to fibres of a given diameter, the small spread would indicate that these potentials also are derived from more than one fibre and that their different propagation velocities are synchronized as in the present experiments at threshold stimulation in the human biceps.

At weak *voluntary effort*, DENSLOW and HASSETT (1943) found propagation velocities varying between 1.3 and 12.5 m/sec. This large variation can, however, not be considered an expression of the true range of velocities within the muscle. The site of innervation may have been situated between the two electrodes used for leading off and the values found may for this reason simulate higher velocities than actually occur. But even if three or more electrodes are used for leading off, whereby this error could be excluded, we found in measurements of the propagation velocity of voluntarily activated fibres a variation by far exceeding that obtained with weak electrical stimulation. This difference must be due to the spatial dispersion of the site of innervation for simultaneously activated fibres; the spikes recorded at the different electrodes not necessarily arising from the same fibres of the motor unit.

Summary.

The arrival time of muscle action potentials evoked by direct local stimulation was determined in 3 to 5 points of the brachial biceps.

The stimulus artifact was reduced by stimulating with a bipolar electrode via a doubly screened transformer with the screen around the secondary coil connected to the muscle.

It was ascertained that potentials from the same few fibres were picked up at the different electrodes as indicated by an identical threshold for the steep potentials of short duration.

The propagation velocity was constant over 10—11 cm muscle length investigated and, therefore, the muscle fibres must pass uninterrupted over this distance.

In ten subjects the propagation velocity varied between 3.3 and 5.2 m/sec. and averaged 4.02 ± 0.13 m/sec. (36.5° C.). In different regions of the same muscle it varied up to 15 per cent. There was no significant difference in the velocity over muscles from subjects of different age (3—74 years). The small variation in propagation velocity in spite of large differences in fibre circumference (2.5—3 times, 19 biopsies) suggest the presence of a mechanism which synchronizes the propagation of the impulses from a small group of simultaneously activated fibres.

Differences in propagation velocity of the order of magnitude found with electrical stimulation can only account for a minor part of the variation in muscle action potential duration obtained with random leads at voluntary effort.

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References.

Buchthal, F., C. Guld and P. Rosenfalck, Action potential parameters in normal human muscle and their dependence on physical variables. Acta Physiol. Scand. 1954. *32*. 200—218.

Buchthal, F., C. Guld and P. Rosenfalck, Innervation zone and propagation velocity in human muscle. Acta Physiol. Scand. 1955 in press.

Denslow, J. S. and C. C. Hassett, The polyphasic action currents of the motor unit complex. Amer. J. Physiol. 1943. *139*. 652—660.

Eccles, J. C. and W. J. O'Connor, Responses which nerve impulses evoke in mammalian striated muscles. J. Physiol. 1939. *97*. 44—102.

Håkansson, C. H., The propagation velocity of isolated frog muscle fibres. Acta Physiol. Scand. 1954. *31*. Suppl. 114. 22—23.

Jarcho, L. W., C. Eyzaguirre, B. Berman and J. L. Lilienthal, Spread of excitation in skeletal muscle: some factors contributing to the form of the electromyogram. Amer. J. Physiol. 1952. *168*. 446—457.

Jarcho, L. W., B. Berman, R. M. Dowben and J. L. Lilienthal, Site of origin and velocity of conduction of fibrillary potentials in denervated skeletal muscle. Amer. J. Physiol. 1954. *178*. 129—134.

Katz, B. and O. H. Schmitt, Electric interaction between two adjacent nerve fibres. J. Physiol. 1940. *97*. 471—488.

Schwalbe, G. and R. Mayeda, Ueber die Kaliberverhältnisse der quergestreiften Muskelfasern des Menschen. Z. Biol. 1890. *27*. 482—516.

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Pharmacological Effects of Humulone on Cats and Rabbits.

By

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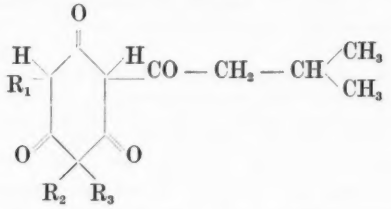
The cones of hops (*Humulus lupulus*. L.) contain two well-known compounds, humulone ($C_{21}H_{30}O_5$) and lupulone ($C_{26}H_{38}O_4$), described more than fifty years ago. They are low-melting, crystalline substances of yellowish colour, easily soluble in all organic solvents and in alkali but practically insoluble in water. The main features of their chemistry were worked out by WÖLLMER (1916, 1925) and WIELAND (1925, 1926), who recognized them as closely related phloroglucinol-derivatives and proposed formulae very similar to the structures I and II, now considered as the most probable ones for humulone and lupulone. These structures, though not completely proved, are in harmony with the results of a considerable amount of recent work on the chemistry of the compounds, including their synthesis. (For references see *Annual Reports of Chemistry*, London 1952.)



Humulone (I): $R_1 = R_2 = Me_2C = CH - CH_2 -$ $\quad R_3 = -OH$
Lupulone (II): $R_1 = R_2 = R_3 = Me_2C = CH - CH_2 -$

The substances extracted from hops have been of particular interest for research in the brewing industry. Although some of the substances have been found to be active against microorganisms *in vitro* (SHIMWELL 1937 and WALKER and PARKER 1937), their pharmacological properties have not been analysed in detail. Lupulone was examined by CHIN and ANDERSON (1950). They estimated LD_{50} for mouse, rat, and guinea pig with different forms of administration and also studied the effects in man of large doses given orally. The antibacterial properties *in vivo* seem to have been uncertain and, furthermore, unfavourable effects such as nausea and vomiting were commonly found. Large doses of lupulone in animals also produced haemorrhages and necrotic foci in liver and kidneys (cf. LEVADITI et al., 1949).

CHIN and ANDERSON (l. c.) also observed that small doses of lupulone, injected intravenously into rabbits and cats, stimulated respiration. They stated that in that respect lupulone in the cat was five times as active as Metrazol but also noticed that it had no awakening activity. SIKORSKI and RUSIECKI (1936), on the contrary, found that both humulone and lupulone were sedative for pigeons and small birds and somewhat less so in mice.

It is a commonly accepted view that dinitrophenols stimulate respiration in animals by means of an increased carbon dioxide production. SÖDERBERG (1952) found that vulpinic acid also exerts its influence upon respiration in a similar way and in another paper SÖDERBERG (1953) demonstrated that usnic acid, which is known to affect oxidative phosphorylations *in vitro* in approximately the same way as the dinitrophenols (JOHNSON et al. 1950), also can accelerate oxidative metabolism *in vivo*. HASSEL (1950) suggested that the symmetrical triketone structure in usnic acid as well as in humulone and lupulone, might account for their antibacterial activity. The present investigation was undertaken to find whether humulone produces the same kind of effects in animals as usnic acid and the dinitrophenols.

Methods.

Cats and rabbits anaesthetized with 5 ml/kg body weight of a solution containing 1 % chloralose and 10 % urethane were used. In addition a group of animals were curarized. For curarization 0.5 ml of a solution containing 3 mg d-tubocurarine chloride (Abbot) per ml was slowly injected intravenously followed by smaller doses during

the experiment if spontaneous respiratory movements or other signs of muscular activity were observed. The effect of humulone was also tested on a few unanaesthetized rabbits.

Blood pressure was recorded from the femoral artery by means of a mercury manometer. Respiration was studied and oxygen consumption estimated by a Krogh spirometer. A Starling pump for artificial respiration connected the curarized animals with the spirometer.

In one group of animals acute partial evisceration was performed in order to study the significance of the liver for the effects provoked by humulone. In order to avoid the difficulties connected with a one stage hepatectomy the liver was left *in situ* with the portal vein and the hepatic artery ligated. Such a preparation behaves in the acute experiment like a hepatectomized animal (cf. RANNEY et al. (1951) and also MANN and MANN (1953), for a general review of liver circulation). The evisceration started with the removal of the intestines, ventricle and the spleen, thus avoiding the surgical shock which generally appears when the portal vein is ligated in an animal without an Eck-fistula. After the liver had been made anemic the suprarenals, and in one experiment also the kidneys were removed. In order to avoid hypoglycemia 5.5 % glucose was administered during the course of the experiments.

Humulone was isolated from the cones of hops by extraction with petrol (40—60° C) and purification via the lead salt and the o-diaminobenzene salt according to WÖLLMER (1925). The preparation obtained had constants, m. p. 58—62° C and $[\alpha]_D^{20}$ —206° (in ethanol, 1.01 g/100 ml), in reasonable agreement with the values for pure humulone reported by WÖLLMER (l. c.), m. p. 65—66.°5 C, $[\alpha]_D^{20}$ —212° (in ethanol). Immediately before use it was dissolved (10 mg/ml) in a phosphate buffer (1 vol. 0.1 *M* Na_3PO_4 and 3 vol. 0.1 *M* Na_2HPO_4) by warming shortly to 60° C. After that the solution was diluted with equal amounts of Ringer. The substance was always administered intravenously.

Results.

In normal anaesthetized cats a few mg of humulone per kg body weight injected intravenously caused a prompt increase in ventilation, sometimes with a few gasps in the beginning. The blood pressure was only slightly influenced. The depth of the narcosis as tested with corneal, pupillary and pinna reflexes was unchanged. In parallel with the hyperventilation the oxygen consumption increased to a level dependent on the dose of humulone given (fig. 1). At the same time the augmented heat production raised the body temperature. In spite of decreased vasomotor tone in the skin, as suggested by the increased skin temperature,

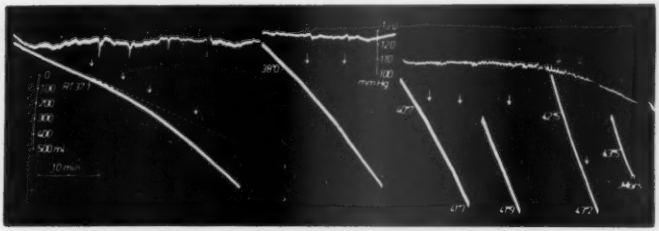


Fig. 1. Cat, 2.2 kg. Chloralose-urethane. Spontaneous respiration. Oxygen consumption and blood pressure. The marks represent the injection of 2 mg humulone. The time interval between the first and second pair of curves is 45 min. The second interval is of 20 min. duration. Thereafter continuous record.

and the appearance of polypnoea (at a rectal temperature of 42° C) and panting, the death from large doses of humulone was probably caused by the hyperthermia which could exceed 45° C.

In order to study whether the increased oxygen consumption was due to increased muscular work or not, humulone was also tested on deeply curarized cats under artificial respiration. As can be seen from fig. 2, d-tubocurarine had no measurable influence upon the effect of humulone on oxygen consumption.

A few minutes after death from a lethal dose of humulone the cats showed a pronounced rigor in their muscles. This effect was also seen in the curarized animals.

Reducing substances were found in the urine after large doses of humulone. In a few cases haematuria was also observed.

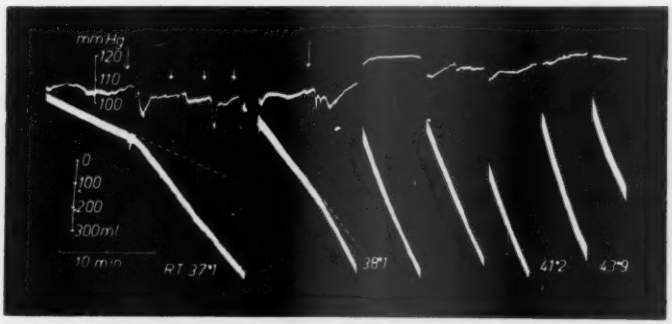


Fig. 2. Cat, 2.6 kg. Chloralose-urethane. Artificial respiration. Oxygen consumption and blood pressure. The big arrows indicate the injections of 15 mg humulone, the small ones represent the administration of 0.6 mg d-tubocurarine chloride. Between each record there is a 15 min. interval.

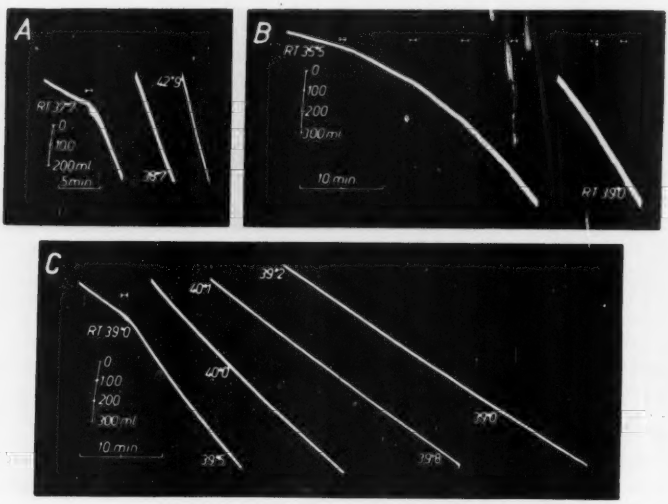


Fig. 3. Chloralose-urethane anaesthesia. Spontaneous respiration. Oxygen consumption. *A*. Cat, 2.5 kg. During the mark 20 mg humulone is injected. Between the first and second record no time interval, between the second and third 35 min. *B*. Cat, 2.2 kg. Continuous record. The effect of consecutive injections of 2 mg humulone into a functionally dehepatized cat. *C*. Rabbit, 2.2 kg. The effect of 20 mg humulone. The first and second interruptions are of 20 min. duration. Thereafter continuous record.

In rabbits the effect of humulone upon body temperature was much weaker and most of the effect had disappeared after four hours. The dose required to give a hundred per cent increase in oxygen consumption was three times larger in the rabbits than in the cats. As can be seen from fig. 3 the effect in the rabbit was also of shorter duration than in the cats. This is of interest because the dinitrophenols similarly exhibit much more of an effect in cats than in rabbits, being detoxified very rapidly by rabbit tissue.

The significance of intact liver function for the humulone effect was studied in a series of experiments on partly eviscerated animals. The liver was switched off from the circulation as described above by ligation of the portal vein and the hepatic artery. In such animals humulone had a much stronger effect than in the controls. This can be seen from fig. 4 which also demonstrates that there is a direct proportionality between dose and activity in the functionally dehepatized animal. The quantitative differ-

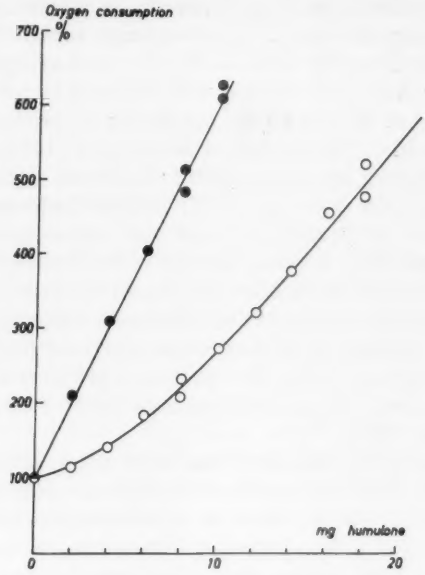


Fig. 4. The effect of different doses of humulone on oxygen consumption. Open circles normal cats. Filled circles functionally dehepatized cats.

ence between cats and rabbits was, however, the same irrespectively of whether the liver was intact or not.

Humulone was also tested on unanaesthetized rabbits. The body temperature was measured and respiration observed. No qualitative differences were found between this group of animals and the anaesthetized ones. No signs of sleepiness or excitation were noticed in these experiments.

Discussion.

The experiments presented support the view that there are important similarities between humulone, usnic acid and the dinitrophenols. These, the long lasting rise of body temperature, the increased oxygen consumption, unaffected by tubocurarine, and the early appearance of rigor are in no case due to increased muscular work. This has been proved by the experiments performed under tubocurarine.

Muscular metabolism must, however, be affected since lethal doses of humulone caused an abnormally severe rigor mortis, which appeared rapidly after death. The onset of rigor was independent of whether the animal was curarized or not. This is of interest because dinitrophenols are known to produce the same effect as shown by HEYMANS and BOUCKAERT (1932). Their finding was explained by ERDÖS (1943) who found a striking parallelism between the depletion of ATP in the muscles and the onset of rigor. JUDAH (1952) confirmed this explanation, when he demonstrated that muscles treated with dinitrophenols have a low content of ATP. The mechanism is, however, complex (BENDALL 1951) and there are substances which retard the establishment of rigor in spite of a low ATP content. This may be illustrated by the finding that usnic acid produces a rapid onset of rigor whereas vulpinic acid seems to have the opposite effect (SÖDERBERG 1953).

The experiments also show that there are quantitative differences in the effect of humulone on different animals. This may be explained by the fact that the detoxifying mechanisms differ. PARKER (1952) also found that 2,4-dinitrophenol is rapidly reduced by rat tissue homogenates. One of the most important organs for the protection against poisons is the liver. It is thus of interest to study the action of humulone on animals with damaged liver function, particularly as RYAN (1951) has shown that the intravenous injection of 2,4-dinitrophenol caused a much more pronounced rise in rectal temperature in dehepatized animals than in normal ones, although blood sugar is increased in the latter but maintains its progressive decrease in the former. Experiments on such animals also showed that a part of the detoxification of humulone may occur in the liver. The removal of the liver functions did not, however, diminish the quantitative difference between cats and rabbits. Whether humulone can be inactivated by other organs than the liver or if the tissues in rabbits and cats have different sensitivity to the substance cannot, however, be concluded from the results presented here.

The experiments have given new evidence that substances chemically quite distinct from dinitrophenols can induce a considerable increase of oxygen consuming processes *in vivo*. Knowledge of several substances of different kinds with approximately the same action on heat production and oxygen consumption in animals should be of value for a more precise understanding

of the mode of action of such substances. An analysis of the relationship between structure and activity will also be published in the near future.

Summary.

1. Humulone, a well known constituent of hops has been tested on cats and rabbits.

2. 3 mg humulone/kg body weight injected intravenously into cats produced instantaneously a 100 % increase in oxygen consumption, followed by a gradual rise in body temperature.

3. Blood pressure was only slightly affected. The amplitude of respiration was increased and when the body temperature reached a level of 42° C temperature polypnoea appeared.

4. Death from lethal doses of humulone was probably caused by the increased body temperature which could exceed 45° C in the cat. After death the animals rapidly developed an abnormally severe rigor mortis. This effect, which was also seen in curarized cats, is similar to the well-known action of the dinitrophenols.

5. Large doses of humulone also produced glycosuria and haematuria.

6. Rabbits were more resistant than cats to humulone.

7. Liverless animals were more sensitive to humulone than those with intact liver.

8. The actions of humulone in animals are on the whole very similar to those of usnic acid and dinitrophenols.

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References.

Annual Reports of Chemistry, London 1952 (p. 189).

Bendall, J. R., J. Physiol. 1951. *114*. 71.

Chin, Y. and H. H. Anderson, Arch. int. pharmacodyn. 1950. *82*. 1.

Erdös, T., Enzimologia 1943. *3*. 51. (Cited from Szent-Györgyi 1947.)

Hassel, C. H., Experientia 1950. *6*. 462.

Heymans, C. and J. J. Bouckaert, Nederl. Tijdschr. Geneeskl. 1932. 3649.

JOHNSON, R. B., G. FELDOTT and H. A. LARDY, Arch. Biochem. 1950. *28*. 317.
JUDAH, J. D., Proc. R. Soc. Med. 1952. *45*. 574.
LEVADITI, C., A. VAISMAN and J. HENRY-EVENO, C. R. Soc. Biol. Paris 1949. *143*. 1474.
MANN, F. C. and F. D. MANN, Ann. Rev. Physiol. 1953. *15*. 473.
PARKER, V. H., Biochem. J. 1952. *51*. 363.
RANNEY, R. E., I. L. CHAIKOFF and E. L. DOBSON, Amer. J. Physiol. 1951. *165*. 588.
RYAN, E. A., Ibidem 1951. *167*. 224.
SHIMWELL, J. L., J. Inst. Brewing 1937. *34*. 191.
SIKORSKI, H. and W. RUSIECKI, Bull. intern. acad. polon. sci. Classe Med. 1936. *73*. 83. (Cited from C. A. 1953. 9280.)
SZENT-GYÖRGYI, A., Chemistry of Muscular Contraction. Acad. Press, New York 1947.
SÖDERBERG, U., Acta physiol. scand. 1952. *27*. 97.
— Ibidem 1953. *28*. 202.
WALKER, T. K. and A. PARKER, J. Inst. Brewing 1937. *43*. 17.
WIELAND, H., Ber. dtsch. chem. Ges. 1925. *58*. 102. 2012.
— Ibidem 1926. *59*. 2352.
WÖLLMER, W., Ibidem 1916. *49*. 780.
— Ibidem 1925. *58*. 672.

1950.

Biol.

ysiol.

Classe

Press,